ULTRASTRUCTURAL INVESTIGATION OF HUMARIACEAE (PEZIZALES, ASCOMYCETES): SPORE ONTOGENY, SEPTAL STRUCTURE, AND PHYLOGENY

By

CHI-GUANG WU

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1991

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to Dr. James W. Kimbrough for this opportunity to study the taxonomy of Discomycetes by using ultrastructural techniques. During the course of this study, his patience in guiding my research and his knowledge of mycology are greatly admired. I am indebted to Dr. H. C. Aldrich for his patience, constructive discussion, and warm encouragement during my studies in electron microcopy and cytochemistry. I am also grateful to the members of my supervisory committee, Drs. Saeed R. Khan, H. C. Kistler, L. H. Purdy.

I also owe appreciation to Dr. Gregory W. Erdos, director of the Electron Microscope Core Laboratory, for his kind suggestions and demonstration of cytochemical and ultrastructural techniques. I also wish to thank all of the staff in the EM Lab, especially Ms. D. Williams.

A heartfelt gratitude is owed to Dr. G. Benny and other colleagues in the Mycology Lab for their encouragement and friendship.

Herein, I would like to acknowledge my former advisor in Taiwan, Dr. Zuei-Ching Chen and Japanese mycologist, Mr. Takashi Matsushima, who always encouraged me during this

reseach since I came to the United States in 1987. Their encouragement and help will be appreciated forever.

Finally, I must thank my wife, Hwung Zue, and my son, James. Without their company and inspiration, this research could not be finished. Any achievements that I may have made in this research belongs to God, because without Him, I could do little.

TABLE OF CONTENTS

page

ACKNOWLEDGEMENTS....ii

LIST OF TABLES....vi

LIST OF FIGURES....vii

ABSTRACT....xxv

GENERAL INTRODUCTION....1

CHAPTER I ASCOSPOROGENESIS IN SELECTED GENERA OF ALEURIEAE....5

Introduction....5
Materials and Methods....9
Results....10
Discussion....16

CHAPTER II ASCOSPOROGENESIS IN SELECTED GENERA OF CILIARIEAE....46

Introduction....46
Materials and Methods....51
Results....52
Discussion....61

CHAPTER III ASCOSPOROGENESIS IN SELECTED GENERA OF LACHNEAE....93

Introduction....93
Materials and Methods....96
Results....97
Discussion....104

CHAPTER IV ASCOSPOROGENESIS IN SELECTED GENERA OF OTIDEAE....138

Introduction....138
Materials and Methods....141
Results....142
Discussion....147

page

CHAPTER V SEPTAL STRUCTURES IN SELECTED GENERA OF ALEURIEAE, LACHNEAE, AND OTIDEAE.....170

Introduction.....170
Materials and Methods.....172
Results.....174
Discussion.....179

CHAPTER VI COMPARATIVE STUDY ON THE SEPTAL DEVELOPMENT BETWEEN ASCOBOLOID AND SCUTELLINIOID TYPES.....198

Introduction.....198
Materials and Methods.....199
Results.....200
Discussion.....202

CHAPTER VII SPORE ONTOGENY, SEPTAL STRUCTURE, CHARACTERS USEFUL IN SHOWING THE PHYLOGENY IN PEZIZALES.....215

Introduction.....215
Description.....216
Discussion.....222

SUMMARY.....249

BIBLIOGRAPHY.....251

BIOGRAPHICAL SKETCH.....261

LIST OF TABLES

page

Table 4.1 Comparison of nuclear number in paraphyses of genera in Humariaceae and Ascobolaceae.....151

Table 5.1 Comparison of pore size in the ascal base and ascogenous hypha from selected species of Aleurieae and Lachneae.....186

Table 5.2 A combination of septal structural data and species studied in Rifai's system...187

Table 7.1 Spore ontogeny, septal structure and the genera studied in the Pezizales (Ascomycetes).....233

Table 7.2 Selected genera of Humariaceae in Rifai's classification system.....234

Table 7.3 The common characters found in four subgroups of Humariaceae, after this study.....235

Table 7.4 The genera that produce globose and smooth ascospores.....236

Table 7.5 The spore wall characters found in pyrophilic species.....237

Table 7.6 Some phylogenetic characters in Humariaceae.....238

LIST OF FIGURES

page

Figure 1.1 Aleuria aurantia. A young ascospore with early secondary wall material deposition.....25

Figure 1.2 Aleuria aurantia. Secondary wall material deposited on primary wall surface.....25

Figure 1.3 Aleuria aurantia. Early formation of epispore layer on primary wall surface...25

Figure 1.4 Aleuria aurantia. Stacks of ER in the epiplasm of ascal tip.....25

Figure 1.5 Aleuria aurantia. A young ascospore with dilated perisporic sac.....27

Figure 1.6 Aleuria aurantia. A mass globule in the perisporic sac.....27

Figure 1.7 Aleuria aurantia. Mass globules in the perisporic sac.....27

Figure 1.8 Aleuria aurantia. Granular matrix in the perisporic sac.....27

Figure 1.9 Aleuria aurantia. Epispore formation on the surface of primary wall.....29

Figure 1.10 Aleuria aurantia. The second layer of epispore formed in the perisporic sac.....29

Figure 1.11 Aleuria aurantia. Detailed view of the epispore layers.....29

Figure 1.12 Aleuria aurantia. Two layered epispore in a maturing ascospore.....29

Figure 1.13 Aleuria aurantia. ER stacks and mitochondria clusters in the epiplasm of ascal tip.....31

Figure 1.14 Aleuria aurantia. Mitochondria and globular bodies surrounded by microtubules in the epiplasm.....31

Figure 1.15 Aleuria aurantia. Dense material and ER stacks in the epiplasm.....31

Figure 1.16 Aleuria aurantia. Dense material within the ascal tip.....31

Figure 1.17 Aleuria aurantia. ER stacks and an ascospore in the ascus.....33

Figure 1.18 Aleuria aurantia. Gradual condensation in the perisporic sac.....33

Figure 1.19 Aleuria aurantia. Mature ascospore wall..33

Figure 1.20 Aleuria aurantia. Detailed view of spore wall structure.....33

Figure 1.21 Aleuria aurantia. Eight ascospores in an ascus.....35

Figure 1.22 Aleuria aurantia. Cellular inclusions of paraphyses.....35

Figure 1.23 Aleuria aurantia. Condensed graules in the perisporic sac.....35

Figure 1.24 Aleuria aurantia. Mature ascospore with two lipid guttules and a nucleus.....35

Figure 1.25 Aleuria aurantia. Mature reticulate ascopsores.....35

Figure 1.26 Octospora euchroa. Spore delimiting membranes in a young ascus.....37

Figure 1.27 Octospora euchroa. Dark and white alternating bands in a spore delimiting membrane.....37

Figure 1.28 Octospora euchroa. Elastic primary wall of a young ascospore.....37

Figure 1.29 Octospora euchroa. An elongate ascospore with early secondary wall deposition.....37

Figure 1.30 Octospora euchroa. Spine-like epispore precursors.....37

Figure 1.31 Octospora euchroa. Epispore showing zebra-like arrangement of fibrils.....37

Figure 1.32 Octospora euchroa. Detailed view of tubular epispore precursors.....39

Figure 1.33 Octospora euchroa. Gradual condensation in the perisporic sac.....39

Figure 1.34 Octospora euchroa. Translucent zone and degenerated perisporic sac.....39

Figure 1.35 Octospora euchroa. An epispore-covered, smooth ascospore.....39

Figure 1.36 Octospora euchroa. Detailed view of a mature ascospore wall.....39

Figure 1.37 Pulvinula convexella. A young ascospore enclosed by primary wall.....41

Figure 1.38 Pulvinula convexella. Primary wall deposited between two spore delimiting membranes.....41

Figure 1.39 Pulvinula convexella. An early stage of secondary wall material deposited in the perisporic sac.....41

Figure 1.40 Pulvinula convexella. Secondary wall material directly deposited on the surface of primary wall.....41

Figure 1.41 Pulvinula convexella. An epispore formed in the secondary wall material.....43

Figure 1.42 Pulvinula convexella. Detailed view of the precursors of epispore 43

Figure 1.43 Pulvinula convexella. Detailed view of mature epispore.....43

Figure 1.44 Pulvinula convexella. Mature spore surface ornamented with rodlet-like furrows.....43

Figure 1.45 Pulvinula convexella. Detailed view of minisculptured surface of ascospore.....43

Figure 1.46 Comparison of spore wall development in Aleuria, Octospora, and Pulvinula of the tribe Aleurieae.....45

Figure 2.1 Cheilymenia stercorea. Nuclei and spore delimiting membranes.....68

Figure 2.2 Cheilymenia stercorea. Spore delimiting membrane connected with ascal plasma membrane.....68

Figure 2.3 Cheilymenia stercorea. Young ascospores..68

Figure 2.4 Cheilymenia stercorea. Fibrillar primary wall.....68

Figure 2.5 Cheilymenia stercorea. A young ascospore with expanded perisporic sac.....68

Figure 2.6 Cheilymenia stercorea. Perisporic sac and secondary wall material.....68

Figure 2.7 Cheilymenia stercorea. Condensation of secondary wall material in the perisporic sac.....70

Figure 2.8 Cheilymenia stercorea. Epispore and condensed secondary wall material.....70

Figure 2.9 Cheilymenia stercorea. Differentiation of translucent zone in the perisporic sac...70

Figure 2.10 Cheilymenia stercorea. Perisporic sac separated by a translucent zone.....70

Figure 2.11 Cheilymenia stercorea. Reticulate condensed material in the perisporic sac.....70

Figure 2.12 Cheilymenia stercorea. Lamellate epispore and degenerated perisporic sac.....70

Figure 2.13 Cheilymenia stercorea. An ascospore enclosed by a spore sheath.....72

Figure 2.14 Cheilymenia stercorea. Detailed view of spore sheath and spore wall structures...72

Figure 2.15 Cheilymenia coprinaria. Fibrillar globular body and reticulate condensed material in the perisporic sac.....72

Figure 2.16 Cheilymenia coprinaria. Spore sheath and mature ascospore.....72

Figure 2.17 Coprobia granulata. Early formed ascospores in a young ascus.....74

Figure 2.18 Coprobia granulata. A young ascospore....74

Figure 2.19 Coprobia granulata. A thin primary wall enclosed by two spore delimiting membranes.....74

Figure 2.20 Coprobia granulata. Early condensation in the perisporic sac.....74

Figure 2.21 Coprobia granulata. Precursors of epispore.....76

Figure 2.22 Coprobia granulata. Smooth epispore and perisporic sac.....76

Figure 2.23 Coprobia granulata. A small lipid body presentin the sporoplasm.....76

Figure 2.24 Coprobia granulata. Early formation of striated ornamentations in the perisporic sac.....76

Figure 2.25 Coprobia granulata. Detailed view of the perisporic sac.....78

Figure 2.26 Coprobia granulata. Gradual condensation in the perisporic sac.....78

Figure 2.27 Coprobia granulata. Reticulate epispore and striated ornaments in the perisporic sac.....78

Figure 2.28 Coprobia granulata. A translucent zone adjacent to the epispore.....78

Figure 2.29 Coprobia granulata. Uneven condensation in the perisporic sac.....78

Figure 2.30 Coprobia granulata. A spore sheath separated from ascospore by a translucent zone.....78

Figure 2.31 Scutellinia scutellata. A haploid nucleus enclosed by spore delimiting membrane....80

Figure 2.32 Scutellinia scutellata. A young ascospore enclosed by primary wall.....80

Figure 2.33 Scutellinia scutellata. Ascospores in an ascus.....80

Figure 2.34 Scutellinia scutellata. Electron dense secondary wall material deposited on primary wall.....80

Figure 2.35 Scutellinia scutellata. Primary wall and perisporic sac.....82

Figure 2.36 Scutellinia scutellata. Precursors of epispore.....82

Figure 2.37 Scutellinia scutellata. Condensed granules in the perisporic sac.....82

Figure 2.38 Scutellinia scutellata. Uneven condensation in the perisporic sac.....82

Figure 2.39 Scutellinia scutellata. Irregular, condensed material and less opaque, globular area in the perisporic sac.....82

Figure 2.40 Scutellinia scutellata. Detailed view of the perisporic sac.....82

Figure 2.41 Scutellinia scutellata. Detailed view of the spore wall structures.....82

Figure 2.42 Scutellinia scutellata. Vacuolation in the perisporic sac.....84

Figure 2.43 Scutellinia scutellata. Ornaments of ascospore.....84

Figure 2.44 Scutellinia scutellata. Translucent bands at the bases of ornaments.....84

Figure 2.45 Scutellinia pennsylvanica. A young ascospore enclosed by primary wall.....86

Figure 2.46 Scutellinia pennsylvanica. Fibrillar bodies in a young ascospore.....86

Figure 2.47 Scutellinia pennsylvanica. Perisporic sac and primary wall.....86

Figure 2.48 Scutellinia pennsylvanica. Uneven condensation in the perisporic sac.....86

Figure 2.49 Scutellinia pennsylvanica. Less opaque areas in the perisporic sac.....88

Figure 2.50 Scutellinia pennsylvanica. electron dense fibrils in the perisporic sac.....88

Figure 2.51 Scutellinia pennsylvanica. Condensed material and globular, less opaque areas in the perisporic sac.....88

Figure 2.52 Scutellinia pennsylvanica. Vacuolation in the perisporic sac.....88

Figure 2.53 Scutellinia pennsylvanica. Detailed view of spore ornaments.....90

Figure 2.54 Scutellinia pennsylvanica. Translucent bands and fibrillar structures in the spore ornaments.....90

Figure 2.55 Comparison of spore wall development in the genera Cheilymenia, Coprobia, and Scutellinia of the tribe Cillarieae.....92

Figure 3.1 Mycolachnea hemisphaerica. A young ascospore enclosed by primary wall.....111

Figure 3.2 Mycolachnea hemisphaerica. Vacuolation in the epiplasm.....111

Figure 3.3 Mycolachnea hemisphaerica. Primary wall deposited between two spore delimiting membranes.....111

Figure 3.4 Mycolachnea hemisphaerica. Crystal-like inclusion body in the sporoplasm.....111

Figure 3.5 Mycolachnea hemisphaerica. Granular secondary wall material in the perisporic sac.....111

Figure 3.6 Mycolachnea hemisphaerica. Membrane-bound bodies in the sporoplasm.....111

Figure 3.7 Mycolachnea hemisphaerica. A globualr body released from sporoplasma membrane into primary wall.....111

Figure 3.8 Mycolachnea hemisphaerica. A young ascospore surrounded by a less-expanded perisporic sac.....113

Figure 3.9 Mycolachnea hemisphaerica. Detailed view of the perisporic sac.....113

Figure 3.10 Mycolachnea hemisphaerica. Secondary wall material condensed into two different zones in the perisporic sac.....113

Figure 3.11 Mycolachnea hemisphaerica. Granular condensation in the inner zone of perisporic sac.....113

Figure 3.12 Mycolachnea hemisphaerica. Condensed granules in the perisporic sac.....113

Figure 3.13 Mycolachnea hemisphaerica. Spore ornaments formed in the perisporic sac...113

Figure 3.14 Mycolachnea hemisphaerica. Clumps of secondary wall material.....115

Figure 3.15 Mycolachnea hemisphaerica. Spore ornaments covered by spines.....115

Figure 3.16 Mycolachnea hemisphaerica. Wall structures in a mature ascospore.....115

Figure 3.17 Mycolachnea hemisphaerica. Radiate structures in the primary wall.....115

Figure 3.18 Mycolachnea hemisphaerica. Spore ornaments covered by small spines.....115

Figure 3.19 Mycolachnea hemisphaerica. Detailed view of spore ornaments.....115

Figure 3.20 Mycolachnea hemisphaerica. Crystal-like inclusion bodies and dumbbell body in the sporoplasm.....117

Figure 3.21 Mycolachnea hemisphaerica. Crystal-like inclusion body in the nucleus.....117

Figure 3.22 Mycolachnea hemisphaerica. Dumbbell body attached to outer nuclear membrane.....117

Figure 3.23 Mycolachnea hemisphaerica. Detailed view of crystal-like inclusion body in the sporoplasm.....117

Figure 3.24 Mycolachnea hemisphaerica. Crystal-like inclusion bodies released from nucleus...117

Figure 3.25 Mycolachnea hemisphaerica. Detailed view of crystl-like inclusion body in the nucleus.....117

Figure 3.26 Mycolachnea hemisphaerica. ER associated with crystal-like inclusion body.....119

Figure 3.27 Mycolachnea hemisphaerica. Multivesicular bodies and crystal-like inclusion bodies associated with ER.....119

Figure 3.28 Mycolachnea hemisphaerica. Degenerating crystal-like inclusion body.....119

Figure 3.29 Mycolachnea hemisphaerica. Degenerating crystal-like inclusion body and, circular chain structures.....119

Figure 3.30 Mycolachnea hemisphaerica. Dumbbell body associated with primary wall.....119

Figure 3.31 Sphaerosporella brunnea. Meiotic nucleus in the ascus.....121

Figure 3.32 Sphaerosporella brunnea. A young ascospore enclosed by primary wall.....121

Figure 3.33 Sphaerosporella brunnea. Primary wall delimited by two unit membranes.....121

Figure 3.34 Sphaerosporella brunnea. A young ascospore with slightly expanded perisporic sac.....121

Figure 3.35 Sphaerosporella brunnea. Secondary wall material deposited in the perisporic sac.....121

Figure 3.36 Sphaerosporella brunnea. Precursors of epispore.....123

Figure 3.37 Sphaerosporella brunnea. Two layers of epispore.....123

Figure 3.38 Sphaerosporella brunnea. Detailed view of spore wall structures.....123

Figure 3.39 Sphaerosporella brunnea. Gradual condensation in the perisporic sac.....123

Figure 3.40 Sphaerosporella brunnea. A translucent zone in the perisporic sac.....123

Figure 3.41 Sphaerosporella brunnea. Detailed view of peripsoric sac and translucent zone.....123

Figure 3.42 Sphaerosporella brunnea. Spine-like structures forming in the primary wall.....123

Figure 3.43 Sphaerosporella brunnea. Gradual condensation in the perisporic sac.....125

Figure 3.44 Sphaerosporella brunnea. Changes in the primary wall.....125

Figure 3.45 Sphaerosporella brunnea. A grazing section of spine layers in the primary wall.....125

Figure 3.46 Sphaerosporella brunnea. Detailed view of the spine layers in the primary wall.....125

Figure 3.47 Sphaerosporella brunnea. An ascospore enclosed by a degenerated perisporic sac.....127

Figure 3.48 Sphaerosporella brunnea. A spherical and smooth ascospore.....127

Figure 3.49 Sphaerosporella brunnea. A mature ascospore and spore sheath.....127

Figure 3.50 Sphaerosporella brunnea. An epispore-exposed ascospore.....127

Figure 3.51 Trichophaea paludosa. A young ascospore enclosed by spore delimiting membranes.....129

Figure 3.52 Trichophaea paludosa. Primary wall of ascospore.....129

Figure 3.53 Trichophaea paludosa. A young ascospore enclosed by primary wall.....129

Figure 3.54 Trichophaea paludosa. A newly formed perisporic sac.....129

Figure 3.55 Trichophaea paludosa. Sporoplasma membrane associated bodies in an ascospore.....129

Figure 3.56 Trichophaea paludosa. Clumps of secondary wall material deposited on primary wall.....129

Figure 3.57 Trichophaea paludosa. Precursors of epispore.....131

Figure 3.58 Trichophaea paludosa. A young ascospore surrounded by secondary wall material....131

Figure 3.59 Trichophaea paludosa. Secondary wall material deposited closer to the epispore.....131

Figure 3.60 Trichophaea paludosa. An ascospore with a nucleus and two guttules.....131

Figure 3.61 Trichophaea paludosa. A layer of newly formed fibrillar primary wall.....131

Figure 3.62 Trichophaea paludosa. Gradual condensation of secondary wall material in the perisporic sac.....133

Figure 3.63 Trichophaea paludosa. Detailed view of the spore wall structures.....133

Figure 3.64 Trichophaea paludosa. Early formed spore ornaments.....133

Figure 3.65 Trichophaea paludosa. Spore ornament loosely attached to epispore.....133

Figure 3.66 Trichophaea paludosa. Pitted ornaments on the epispore.....133

Figure 3.67 Trichophaea paludosa. Detailed view of the spore ornament.....133

Figure 3.68 Trichophaea paludosa. A mature ascospore.....135

Figure 3.69 Trichophaea paludosa. Spore ornament covered by spine-like structures.....135

Figure 3.70 Trichophaea paludosa. An ascospore enclosed by a broken investing membrane.....135

Figure 3.71 Trichophaea paludosa. Ornamented ascospores.....135

Figure 3.72 Trichophaea paludosa. Detailed view of ornaments covered by spine-like structures.....135

Figure 3.73 Comparison of spore wall development in Mycolachnea, Sphaerosporella and Trichophaea.....137

Figure 4.1 Tarzetta cupularis. Spore delimiting membranes and haploid nuclei.....153

Figure 4.2 Tarzetta cupularis. A spore delimiting membrane connected with ascal plasma membrane.....153

Figure 4.3 Tarzetta cupularis. Spore delimiting membranes and haploid nuclei.....153

Figure 4.4 Tarzetta cupularis. Newly formed perisporic sac.....153

Figure 4.5 Tarzetta cupularis. Young ascospores.....153

Figure 4.6 Tarzetta cupularis. Electron dense initial wall.....155

Figure 4.7 Tarzetta cupularis. Detailed view of the electron dense initial wall.....155

Figure 4.8 Tarzetta cupularis. Precursors of epispore.....155

Figure 4.9 Tarzetta cupularis. Detailed view of precursors of epispore.....155

Figure 4.10 Tarzetta cupularis. The deposition of secondary wall material in the perisporic sac.....157

Figure 4.11 Tarzetta cupularis. Detailed view of the spore wall strucuture.....157

Figure 4.12 Tarzetta cupularis. Ascospores with two guttules.....157

Figure 4.13 Tarzetta cupularis. Epispore covered by a thin layer of secondary wall material....157

Figure 4.14 Tarzetta cupularis. An mature ascospore..159

Figure 4.15 Tarzetta cupularis. Detailed view of a mature ascospore wall.....159

Figure 4.16 Tarzetta gaillardiana. Spore delimiting membranes and haploid nuclei.....161

Figure 4.17 Tarzetta gaillardiana. Spore delimiting membranes.....161

Figure 4.18 Tarzetta gaillardiana. Perisporic sac and electron dense initial wall.....161

Figure 4.19 Tarzetta gaillardiana. Electron dense globular bodies in the perisporic sac....161

Figure 4.20 Tarzetta gaillardiana. Formation of the epispore.....163

Figure 4.21 Tarzetta gaillardiana. The epispore precursors.....163

Figure 4.22 Tarzetta gaillardiana. Globular bodies in the perisporic sac.....163

Figure 4.23 Tarzetta gaillardiana. Detailed view of spore wall structures.....163

Figure 4.24 Tarzetta gaillardiana. Secondary wall material deposited in the perisporic sac.....163

Figure 4.25 Tarzetta gaillardiana. Mature ascospores.....165

Figure 4.26 T. gaillardiana. Nuclei with condensed chromatin in the cells of paraphyses.....165

Figure 4.27 T. gaillardiana. Nuclei in the cells of paraphyses tips.....165

Figure 4.28 Tarzetta cupularis. A crystal body in the paraphysis cell.....165

Figure 4.29 Tarzetta cupularis. Detailed view of crystal body.....165

Figure 4.30 Otidea alutacea var. microspora. Two nuclei in the cell of a paraphysis.....165

Figure 4.31 Otidea alutacea var. microspora. Ascospores covered by reticulate ridges.....167

Figure 4.32 Otidea onotica. Ascospores covered by reticulate ridges.....167

Figure 4.33 Otidea onotica. Spore ornaments with fibrillar contents.....167

Figure 4.34 Otidea grandis. Ascospores with reticulate ridges.....167

Figure 4.35 Otidea leporina. Smooth ascospores covered by membraneous sheath.....167

Figure 4.36 Comparative spore ontogeny between Tarzetta and Otidea.....169

Figure 5.1 Aleuria aurantia. Septal plugging in the ascogenous hyphae.....189

Figure 5.2 Aleuria aurantia. Detailed view of septal strucuture of ascogenous hypha....189

Figure 5.3 Aleuria aurantia. Septal structure in the pore of ascal base.....189

Figure 5.4 Octospora euchroa. Septal structure of ascal base.....189

Figure 5.5 Pulvinula convexella. Lamellate structure in the pore plug of ascogenous hypha.....189

Figure 5.6 Pulvinula convexella. Lamellate structure of pore plug in a young ascus.....189

Figure 5.7 Pulvinula convexella. Septal structure in the mature ascus base.....189

Figure 5.8 Sphaerosporella brunnea. Pore plugging at the young ascal base.....191

Figure 5.9 Sphaerosporella brunnea. Detailed view of pore plugging structure of young ascal base.....191

Figure 5.10 Sphaerosporella brunnea. Septal structure in the ascogenous hypha.....191

Figure 5.11 Sphaerosporella brunnea. A hemispherical band at the ascal base.....191

Figure 5.12 Sphaerosporella brunnea. Translucent bands found in the ascal plugging structure....191

Figure 5.13 Sphaerosporella brunnea. A hemispherical band found in mature ascal base.....191

Figure 5.14 Sphaerosporella brunnea. Septal structure in the vegetative hyphae.....191

Figure 5.15 Mycolachnea hemisphaerica. Two septal plugging structures in the ascal base....193

Figure 5.16 Mycolachnea hemisphaerica. Double translucent zone in the septal structure of ascogenous hypha.....193

Figure 5.17 Mycolachnea hemisphaerica. Septal structure in a young ascal base.....193

Figure 5.18 Mycolachnea hemisphaerica. Septal structure of ascal base.....193

Figure 5.19 Mycolachnea hemisphaerica. Septal structure found in the excipular hyphae.....193

Figure 5.20 Mycolachnea hemisphaerica. Septal structure found in the paraphysis cell...193

Figure 5.21 Trichophaea abundans. Septal structure in the ascogenous hyphae.....195

Figure 5.22 Trichophaea abundans. Detailed view of the septal structure of ascogenous hypha.....195

Figure 5.23 Trichophaea paludosa. Septal strucuture of ascogenous hypha.....195

Figure 5.24 Trichophaea paludosa. Septal structure of ascal base.....195

Figure 5.25 Trichophaea abundans. Septal structure of ascal base.....195

Figure 5.26 Trichophaea abundans. A hemispherical and reticulate septal structure of ascal base.....195

Figure 5.27 Trichophaea paludosa. Septal structure in the paraphysis.....195

Figure 5.28 Diagramatic sketch showing different developmental stages of helvelloid, aleurioid and anthracobioid types of septa.....197

Figure 6.1 Ascobolus stictoideus. Septal structure in a crozier.....208

Figure 6.2 Ascobolus stictoideus. Septal structure of ascogenous hypha.....208

Figure 6.3 Ascobolus stictoideus. Septal structure of ascogenous hypha.....208

Figure 6.4 Ascobolus stictoideus. Septal structure of ascogenous hypha.....208

Figure 6.5 Ascobolus stictoideus. Septal structure of ascal base.....208

Figure 6.6 Scutellinia scutellata. Septal structures in ascogenous hypha.....208

Figure 6.7 Scutellinia scutellata. Septal structure of ascogenous hypha.....208

Figure 6.8 Scutellinia scutellata. A translucent torus in the plugging matrix of ascogenous hypha.....210

Figure 6.9 Scutellinia scutellata. Septal structure of ascogenous hypha.....210

Figure 6.10 Scutellinia scutellata. Septal structure in a young ascal base.....210

Figure 6.11 Scutellinia scutellata. Septal structure in a young ascal base.....210

Figure 6.12 Scutellinia scutellata. Septal structure in a young ascal base.....210

Figure 6.13 Scutellinia scutellata. A hemispherical band in the mature ascal base.....210

Figure 6.14 Scutellinia scutellata. Lower band of ascal septal plug removed by hydrogen peroxide.....210

Figure 6.15 Scutellinia scutellata. Lamellate structure and hemispherical band in the pore of ascal base.....210

Figure 6.16 Coprobia granulata. Septal structure of the ascal base.....212

Figure 6.17 Coprobia granulata. Septal structure of the ascal base.....212

Figure 6.18 Coprobia granulata. Septal structure of the mature ascal base.....212

Figure 6.19 Cheilymenia stercorea. Hemispherical bands at the pores of ascal base.....212

Figure 6.20 Cheilymenia stercorea. A zonate hemispherical band of ascal base.....212

Figure 6.21 Comparison of septal development in the anthracobioid, scutellinioid and ascoboloid types.....214

Figure 7.1 Comparative spore ontogeny between gradual condensation (A and B) and direct precipitation (C) types.....240

Figure 7.2 Comparative formation of smooth spore by gradual condensation and direct precipitation types respectively.....242

Figure 7.3 Comparative spore ontogeny with different types of septal structures.....244

Figure 7.4 Comparative septal development in the ascogenous hyphae and ascal bases.....246

Figure 7.5 Phylogenetic arrangement of Humariaceae and other families in Pezizales based on septal development.....248

Abstract of Dissertation Presented to the Graduate school of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

ULTRASTRUCTURAL INVESTIGATION OF HUMARIACEAE (PEZIZALES, ASCOMYCETES): SPORE ONTOGENY, SEPTAL STRUCTURE, AND PHYLOGENY

by

Chi-Guang Wu

August, 1991

Chairman: James W. Kimbrough
Major Department: Plant Pathology

In the taxonomy of Ascomycetes greater ultrastructural emphasis has been placed on apical ascal dehiscence apparatus. Although the operculum provides significant contributions to the delimitation of families and genera, there is still taxonomic confusion in the family Humariaceae, especially the Aleuria-Otidea complex.

Recently, septal structures and spore ontogeny have been found to be consistent characters in Pezizaceae and Ascobolaceae. The primary purpose of this research is to investigate the different types of septal structures and spore ontogeny that may exist in the Humariaceae, and to compare these data with that of other families of Pezizales. To do this, approximately 25 species, representing 18 different genera, were examined with the aid of the electron microscope. Attention was paid to morphology and development of septal structures and spore formation.

Four totally different septal development types were found in Humariaceae and at least seven in the Pezizales. The developmental study on septal structures provided very valuable information on pezizalean evolution. Septal types are thought to be one of the most conservative features in the fungal taxonomy. From the sequence of septal pore development, the relationship among the humariaceous genera is clearly pictured.

Only two basic patterns of spore wall ontogeny were found. One is gradual condensation type (GC) and the other is direct precipitation type (DP). Specific types of septal structures are always related with certain distinct types of spore ontogeny. These two characters are shown to be extremely useful in the study of phylogeny in the Pezizales. Genera of Humariaceae can be separated into four groups: (i) scutellinioid-GC group, their apothecia producing smooth or rough ascospores, mostly forming spore sheath and covered by rooting excipular hairs; (ii) anthracobioid-GC group, their apothecia mostly from burnt ground, producing smooth spores and forming spore sheath too; (iii) aleurioid-GC group, apothecia either covered by hairs or glabrous, ascospores very rough and highly ornamented; and (iv) helvelloid-DP group, apothecia mostly found from burnt ground, producing smooth spores at light

microscope level, not forming spore sheath. A new hypothesis of fungal evolution in Pezizales is proposed.

GENERAL INTRODUCTION

The Humariaceae, recognized by Boudier (1885) and other mycologists (Rifai, 1968; Dennis, 1968), is the largest and most difficult family of operculate discomycetes (Kimbrough, 1970). There has been considerable controversy as to the limits of this taxon, since the characters in this family show continuous patterns of variation. It is also difficult to understand the relationship of Humariaceae to other families in Pezizales.

Rifai (1968) conceived Humariaceae as a broad family, related on one end to Pyronemataceae (a monotypic family) and to Helvellaceae on the other. He also proposed that one part of Humariaceae might have originated from Helvellaceae, based on the color of apothecia and excipular structures. However, most discussions regarding phylogeny both within the order and among the Ascomycetes in general have been speculative (Kimbrough and Curry, 1986b).

Much effort has been made looking for new characters which may be useful in defining the family Humariaceae and understanding the phylogenetic relationship with other families of Pezizales. These efforts include the operculate structures of ascal dehiscence (Brummelen, 1981;

Samuelson, 1977, 1978a, 1978b, 1978c), spore ontogeny (Merkus, 1973, 1974, 1975, 1976; Dyby and Kimbrough, 1987; Gibson and Kimbrough, 1988a, 1988b), and septal structures (Curry and Kimbrough, 1983; Kimbrough and Curry, 1985, 1986a, 1986b; Kimbrough and Gibson, 1989).

Generally speaking, there are eight different types of opercula found in the Pezizales, and most of the opercular types are very consistent with the existent families such as Ascobolaceae, Ascodesmidaceae, Helvellaceae, Pezizaceae. However, the major group of Humariaceae, Otidea-Aleuria complex, could not be separated by this character. They all share the Octospora type of operculum (Brummelen, 1981).

As to the character of spore ontogeny, Merkus (1973. 1974, 1975, 1976) classified the secondary wall formation (ornamentation) into seven different types and categorized the general spore formation into eighteen different patterns. Her data suggested that spore ontogeny could not be used as a good character in taxonomy, since different types of spore ontogeny occurred in the same genus. On the contrary, the recent studies in Pezizaceae (Dyby and Kimbrough, 1987) and Helvellaceae (Gibson and Kimbrough, 1988a, 1988b; Kimbrough *et al.*, 1990) showed identical types of spore formation at both generic and familial levels. In these recent published papers, detailed

descriptions and illustrations were strongly convincing. A reconfirmation of Merkus' (1973, 1974, 1975, 1976) data is needed.

After Kimbrough and his colleagues (1983, 1985) worked out the septal studies in Pezizaceae and Ascobolaceae, septal structures especially from ascogenous hypha and ascal bases were found very consistent at the generic and familial levels and showed potential value in phylogenetic studies in the cup fungi. Later, they published preliminary studies on septal structures in the tribes Aleurieae and Ciliarieae (Humariaceae). Different types of septal structures were recorded in the same tribe (Kimbrough and Curry, 1986a, 1986b). These data suggest again that Humariaceae is a polyphyletic group.

The purpose of this dissertation is to study extensively from selected genera of each tribe of Humariaceae (i) spore ontogeny, (ii) septal structures and their development, and (iii) the phylogenetic relationships among the families of Pezizales based upon ultrastructural features. These sujects will be covered as follows: Ascosporogenesis in selected genera of Aleurieae (Chapter I), Ciliarieae (Chapter II), Lachneae (Chapter III) and Otideae (Chapter VI); septal structures in selected genera of Aleurieae, Lachneae, and Otideae (Chapter V), comparative septal structures of ascoboloid and

scutellinioid types (Chapter VI), and a general summary of the ultrastructure of spore and septa, and their impact on phylogeny in the Pezizales (Chapter VII).

CHAPTER I
ASCOSPOROGENESIS IN SELECTED GENERA OF ALEURIEAE

Introduction

Since Boudier (1907) first erected the family Humariaceae to accommodate those small, sessile terrestrial cup fungi producing hyaline spores, this family has presented one of the major problems in pezizalean systematics. This is due to the occurrence of continuous patterns of variation in several apothecial and spore characters. Some years later, Le Gal (1947) slightly emended this family by considering features of the excipulum. The family contained three tribes, Lachneae, Ciliarieae, and Humarieae, based mainly on the features of hairs and color reaction of paraphyses with iodine. Unfortunately, the family incorporated a very heterogeneous group of fungi with many inconsistent characters. In 1968, Arpin, Eckblad, and Rifai each proposed three different systems of classifying this family. Eckblad (1968) discussed the difficulties in finding consistent criteria on which to separate humariaceous genera into subdivisions. Most of these criteria, including excipular anatomy, hairs, and carotenoids, met with objections. He proposed instead two families, the Pyronemaceae and a new family Otideaceae. The Otideaceae was characterized by glabrous apothecia,

distinctly two-layered excipulum and non-carotenoid hymenium. The following genera were placed in the Otideaceae: Otidea, Geopyxis, Pustulina, Sowerbyella, Ascosparassis. The Pyronemaceae comprised a large group of genera with continuous patterns of variation in many characters. He proposed three phylogenetic lines in this family, among which Pulvinula and Octospora were considered closely related to Iodophanus in its slightly protruding asci, but Aleuria was treated in another line closely related to Anthracobia and Melastiza, based on spore and excipular structures.

Arpin (1968) used pigmentation (carotenoids) as a major character to separate the humariaceous fungi into two groups, Aleuriaceae and Otideaceae. However, Rifai (1968) accepted the Humariaceae as a large family with four tribes, the Otideae, Lachneae, Ciliarieae, and Aleurieae. This concept has been recognized and adopted by Dennis (1968) and Breitenbach and Kranzlin (1984), but Dennis's system differs slightly from Rifai's by putting Otideae together with Pezizaceae. Eckblad (1968) and Korf (1972) placed most taxa of the Humariaceae in the expanded family Pyronemataceae, while Rifai (1968) and Kimbrough (1970, 1989) restricted the Pyronemataceae to small gymnohymenial species. Samuelson's (1978c) data on ascus structure also supported the suggestion.

In this study, the Aleurieae (Humariaceae) of Rifai's (1968) system is chosen as the first group in which to investigate ascosporogenesis. The Aleurieae includes a group of fungi with or without excipular hairs, mostly growing on the ground, and usually with paraphyses that stain green in Melzer's reagent. Morphology of ascospores was thought to be of much value in classification of Pezizales. In this tribe, however, many different types of ascospores are produced from smooth to reticulate, spherical to ellipsoid. Even in the same genus, such as Pulvinula, in which most species have spherical spores, two ellipsoid-spored species occur (Pfister, 1976; Korf and Zhuang, 1984). With the light microscope, Le Gal (1947) studied many species in detail as to the development and patterns of the ornamentation and incorporated the results into her taxonomy. She also tried to determine their chemical composition. Aleuria aurantia (Pers. ex Hook.) Fuckel was one of the species in this tribe illustrated by Le Gal for its ornament development. She treated this species together with Melastiza chateri (W. G. Smith) Boud. and Scutellinia pseudotrechispora (Schroet.) Le Gal, based on the same pattern of spore ornamentation. They all have the following features, (i) a complex ornamentation which contains callose and pectin, (ii) the "perispore" (=perisporic sac) encloses "masses globuleuses" which come

from epiplasmic vacuoles, and (iii) at the later stage, both "perispore" and "masses globuleuses" disappear.

With the transmission electron microscope, Merkus (1974) found sporogenesis in Pyronemataceae (sensu Eckblad 1968) as very similar and without much diversity as concluded by Le Gal (1947). Merkus's data did not support Le Gal's observations, since no ultrastructural evidence was presented for the presence of "masses globuleuses" or for sporal origin of the substance of the ornamentation constituting the secondary wall. In sharp contrast to her previous paper (Merkus, 1974), Merkus (1976) summarized 18 different types of ascosporogenesis and treated Aleuria and Octospora as having the Lamprospora dictydiola type. Relative to secondary wall formation, however, Aleuria and Octospora were thought to be different in having separate types. Even though Merkus (1973, 1974, 1975, 1976) observed many different genera of operculate fungi, some important genera such as Pulvinula were still not covered. Most of Merkus's illustrations were of certain spore stages and further thorough investigations are still needed on complete spore ontogeny.

Merkus's (1974) preliminary results already showed that spore ontogeny was different for smooth and ornamented spores. Through a series of observations of spore ontogeny on Pezizaceae and Helvellaceae (Dyby and Kimbrough, 1987;

Gibson and Kimbrough, 1988a, 1988b; Kimbrough *et al.*, 1990; Merkus, 1975, 1976), the type of ascosporogenesis is very consistent at the generic and familial levels, even though the same pattern could be shared with other families. Consequently, ascosporogenesis in a heterogeneous family such as Humariaceae needs to be studied genus by genus. These data are expected to shed some light on the phylogenetic relationship between the genera. We also anticipate finding a direction to rearrange the Humariaceae (sensu Rifai, 1968).

Material and Methods

Field specimens were processed for transmission electron microscope study following the procedures described by Curry and Kimbrough (1983). The following collections were observed: Aleuria aurantia (Pers. per Hook.) Fckl. Gainesville, Alachua Co., Florida, FLAS F53706; Octospora euchroa (Karst.) Berthet. (unaccessioned); Pulvinula convexella (Karst.) Pfister; on soil, Gainesville, Alachua Co., Florida, FLAS F55444. Plastic blocks were sectioned on an LKB Huxley ultramicrotome with a diamond knife. After poststaining with uranyl acetate and lead citrate, the sections were examined at 60 kv, on a JEOL 100-CX electron microscope. In order to observe sporoplasm in mature ascospores clearly, some of the sections, before being examined by

electron microscope, were treated by 1% hydrogen peroxide for 1-5 minutes following regular poststaining procedure.

Results

Aleuria aurantia

The early stage of ascosporogenesis is very consistent with other genera of Pezizales. An electron translucent primary wall is enclosed by spore delimiting membranes and the outer membrane (=investing membrane) later expands to become a perisporic sac (Figs. 1.1, 1.46) within which some electron-dense secondary wall material is deposited. At the same time, wall material with similar electron density is found inside the margin of the primary wall (Fig. 1.1, arrow head). Large lipid drops also appear at this stage. All of the young spores are surrounded by giant vacuoles and the epiplasm is expelled to the periphery of the ascus. In the epiplasm, much endoplasmic reticulum is found, especially around the ascal tip (Figs. 1.4, 1.13-1.17). The endoplasmic reticulum closest to the ascal wall is very electron-dense (Figs. 1.1, 1.4, 1.15, 1.17). As the outer investing membrane swells outward, electron-dense secondary wall clumps in the perisporic sac gradually become evenly distributed onto the primary wall (Figs. 1.2, 1.3). Vacuoles, which are frequently found invaginated by investing membranes, result in a reticulate appearance of the perisporic sac (Fig. 1.3). In addition, concentric

membrane complexes are often seen adjacent to the endoplasmic reticulum in the ascal tip (Fig. 1.4). Precursors of the epispore wall which are present on the primary wall as dotted chain appear derived from secondary wall material in the perisporic sac (Fig. 1.3). When these epispore precursors are converted into a layer of radially striate fibrils, secondary wall material starts condensing into dark granules (Figs. 1.5-1.8). Some globular masses are found attached to the epispore (Figs. 1.5-1.7), which are surrounded by electron-dense granules (Figs. 1.6 & 1.7, arrows). The globular masses are electron-opaque globules that are present in the perisporic sac for a short time and then disappear in the matrix leaving blank spots (Fig. 1.7, asterisk). At this stage, the perisporic sac is highly distended and filled with a granular matrix (Figs. 1.5, 1.7). As vacuoles of the epiplasm enlarge, the perisporic sac recedes and the granular matrix within becomes more dense and electron opaque (Fig. 1.8).

After the first layer of the epispore wall (EP1) is formed, secondary wall material starts to condense into electron-dense granules. These congeal later into a dark band and leave a space above the EP1 where the formation of secondary epispore layer (EP2) occurs (Figs. 1.9-1.11). After the second epispore layer is formed, condensed granules continue to migrate toward the epispore leaving a

space between the epispore and a dark periclinal band (Fig. 1.11). In mature spores, there are only two layers of the epispore (Figs. 1.12, 1.18, 1.20). The space on the top of EP2 is finally diminished and is occupied by secondary wall (Fig. 1.20).

In the epiplasm of the ascus apex, a large cluster of mitochondria is surrounded by smooth endoplasmic retiuclum (Fig. 1.13) from which membrane-bound globular bodies are produced (Figs. 1.13, 1.14, hollow arrow). A number of cross-sectioned microtubules are found around these mitochondria (Fig. 1.14, arrows), indicating that they cross perpendicularly. Similar microtubules are also found around the ER stack (Figs. 1.13, 1.14). The endoplasmic reticulum at the inner periphery of the ascal wall contains very dense material (Fig. 1.15, arrows) which later appear either in the cytoplasm around the endoplasmic reticulum (Fig. 1.16, arrows) or near the plasma membrane at the ascal tip (Fig. 1.16). Secondary wall deposition has progressed well by this time. As spore wall deposition continues, a number of changes occur in the epiplasm, and the endoplasmic reticulum with dense cytoplasmic contents is no longer detected (Fig. 1.16).

After formation of the epispore layer, secondary wall material within the perisporic sac condenses into large spherical dense bodies (Figs. 1.17, 1.18, 1.23). Electron

dense reticulate structures are also formed at each end of the ascospore which later become apiculi. Near final maturation, the dense bodies gradually attach to and accumulate on the epispore layer to form ornaments (Figs. 1.18-1.20, 1.23-1.25).

In the ascus, spore wall formation is synchronized in each ascospore (Fig. 1.21). The paraphyses are longer than asci (Fig. 1.21) and contain numerous dense bodies which are assumed to be carotenoid pigmented bodies since they are prominent at the light microscope level (Fig. 1.22). The cytoplasm, especially the lipid bodies of mature ascospores, is very often darkly stained (Fig. 1.25), but after bleaching with hydrogen peroxide two large lipid bodies and one nucleus are revealed (Figs. 1.23-1.24).

Octospora euchroa

Spore delimiting membranes (SDM) with alternating dark and white bands (Figs. 1.26, 1.27) occur in the young ascus. These dark bands contain electron-dense material at various intervals (Fig. 1.27, arrows). After the enclosure of each haploid nucleus, an electron-translucent primary wall is formed between SDMs (Fig. 1.28). The primary wall may be very elastic in this stage since various shapes of young spores are found. The outer investing membranes later expand to form a perisporic sac in which granular,

secondary wall material is deposited. In addition, electron-dense clumps are also detected (Fig. 1.29). These clumps later disappear and become part of the matrix within the perisporic sac. Through condensation of secondary wall material, numerous spinelike precursors of the epispore layer are formed and become arranged somewhat evenly on the primary wall (Figs. 1.29-1.30). These spinelike structures are radially arranged in cross section (Fig. 1.30), but are tubular when sectioned tangentially (Fig. 1.32, arrow heads), and their tips adhere randomly forming an appearance of cerebral furrows on the primary wall (Figs. 1.31, 1.32). Later, secondary wall material in the perisporic sac starts condensing into less stained spots (Fig. 1.33). However, a translucent zone is differentiated at this stage which separates the perisporic sac from epispore layers (Figs. 1.34-1.35). The separated perisporic sac degenerates leaving a very smooth ascospore (Fig. 1.36). The primary wall of a mature ascospore is ornamented with radiate, but branched structures (Figs. 1.35-1.36).

Pulvinula convexella

As in the previous genera, a fibrillar primary wall is enclosed by spore delimiting membranes (Figs. 1.37, 1.38). The outer investing membrane soon dilates to form a

perisporic sac in which fibrillar material is deposited (Figs. 1.39, 1.40). The initial secondary wall material condenses and becomes distributed in clumps around the primary wall (Fig. 1.40, asterisks). In this stage, a few lipid bodies are found within the ascospores (Figs. 1.39-1.42). Spinelike precursors of the epispore layer are synthesized from the condensed material in the perisporic sac and are evenly distributed along the primary wall (Figs. 1.41-1.43). Residues of secondary wall material are condensed in clumps onto the thickened epispore layer (Figs. 1.42, 1.43). There appears to be a periclinal arrangement of microfibrils in the epispore at this stage, although projections of the radial precursor fibrils may still be seen (Fig. 1.43). Furrows of rodletlike bodies are observed in tangential sections of the secondary wall (Fig. 1.44). In mature spores, the outer layer of the primary wall nearest to epispore layer becomes electron opaque, the epispore appears zonate, and there is a thin, evenly distributed secondary wall layer which is separated from epispore layers by a permanent translucent zone (Fig. 1.45). The outermost layer of the secondary wall is finely echinulate as a result of condensation of rodlets within the matrix of the perisporic sac (Fig. 1.45, arrow).

Discussion

In this study, three different types of spore wall deposition were found (Fig. 1.46), even though the genera all have been placed in the same group of pigmented cup fungi. First, the spore surface of Pulvinula convexella turns out to have furrowed structures which become appressed and are not visible at the light microscopic level. This type of wall deposition is thought to be closer to the helvelloid group (Gibson and Kimbrough, 1988a, 1988b; unpublished data). Octospora euchroa is a truely smooth-spored species. Its spore wall formation results from the presence of a translucent zone which separates the perisporic sac from the epispore and indirectly interferes with condensed secondary wall material attaching to the epispore. Excluding the presence of the translucent zone, ascosporogenesis of Octospora is generally more similar to Aleuria, because they all produce condensed granules randomly distributed in the perisporic sac.

Spore ontogeny in Octospora euchroa is similar to that shown by Merkus (1976), even though she did not record the presence of a translucent zone and the disintergration of the perisporic sac which closely resembles that of Anthracobia (Merkus, 1974). A radiate striation is also found in the primary wall of Anthracobia. This similarity

correlates with the results from septal studies by Kimbrough and Curry (1986b) in that both possess hemispherical structures in their ascal base and rectangular or hexagonal Woronin bodies around the septal pores of paraphyses and excipular cells.

The appearance of a translucent zone between the epispore layer and secondary wall during the early stage of wall formation is also very pronounced in coprophilous genera such as Ascobolus (Wu and Kimbrough, 1991a, 1991b; Wells, 1972), Cheilymenia, Coprobia (Chapter II), and Ascodesmis (Mims *et al*, 1990).

In the study of A. aurantia, spore formation is exactly the same as was reported by Merkus (1974, 1976). However, these data conflict with the conclusion made by Bellèmere and Melendez-Howell (1976) that in the Pezizales ascospore walls are formed by a similar process leading to a similar structural scheme. Gradual condensation of secondary wall material with electron-opaque clumps within perisporic sac appears to be a major difference between Aleuria/Octospora and Pulvinula (Fig. 1.46). Condensed clumps within the perisporic sac function as bricks used to construct the secondary wall. This type of spore ontogeny has also been found in other humariaceous genera (Scutellinia, Cheilymenia, Mycolachnea, and Trichophaea,

Chapter II and III) and ascoboloid genera (Ascobolus, Wu and Kimbrough, 1991a, 1991b; Saccobolus, unpublished data).

In this study, precursors of the epispore layer appear to come from the secondary wall material within the perisporic sac, which is contrary to the data published by Merkus (1973, 1974, 1975) and Delay (1966). They thought that the epispore was differentiated from the primary wall. In all three genera of aleurieae studied, spinelike precursors formed from granular material within the perisporic sac and adhere in a radial pattern to the primary wall. Nevertheless, the epispore of Aleuria could be divided into two parts, a thicker inner layer with a spinelike arrangement, and a thinner outer layer with periclinal fibrils which appeared to form directly from sedimentation of wall material within the perisporic sac. Octospora and Pulvinula produce smooth or minutely echinulate spores, and the layers of epispore are essentially the same.

Numerous layers of endoplasmic reticulum and the associated electron-dense material are found in the apical area of the ascus in A. aurantia. This phenomenon, also observed by Samuelson (1978b), has not been reported in other genera of cup fungi. Since the electron-dense material disappears after ascospore maturation, it might be involved in spore formation (Figs 1.13, 1.14). Globular

bodies found within the ER-mitochondria complex are of similar size and electron-density as those attached to the epispore in developing spores (Figs 1.5, 1.6). Apparently these are the globular bodies Le Gal (1947) found in the epiplasm of species of Aleuria, Melastiza, and Ciliaria. It has been shown that the endoplasmic reticulum is involved in the elaboration of glycogen, carotenoids and amyloids (Schrantz, 1971). Other globular structures are also found associated with the endoplasmic reticulum. Their function is unknown, but since they contain crystal-like inclusions they are thought to be microbodies. These globular bodies are released later into the epiplasm. Similar bodies could be traced into the perisporic sac in Lasiobolus pilosus (Fr.) Sacc. (Merkus, 1976). These bodies (globular masses) are always surrounded by tiny dense granules. They disappear after secondary wall material coagulates into spherical dense bodies. The chemical composition and function of these mass globules are unknown but their involvment in secondary wall formation is certain. Cytochemical studies of these wall compounds are planned.

Microtubules as observed in the ER complex, differ in diameter from minimicrotubules which were also associated with ER complex and first observed in the haustoria of the entomogenous imperfect fungus Termitaria snyderi Thax (Khan

and Aldrich, 1975). They suggested that synthesized proteins were transferred to smooth endoplasmic reticulum and polymerized to form minimicrotubules. The tubules were thought to be transport vehicles of protein during hyphal growth. In Aleuria, the microtubules are around 25 nm in diameter and they are not only shown around ER, but at the periphery of mitochondria. The real function for these microtubules associated with mitochondria and ER stacks is still an enigma.

An unique character of Octospora is that wall material starts to deposit between spore delimiting membranes before they enclose haploid nuclei. An identical phenomena was also detected in Anthracobia (unpublished data). This provides additional evidence to support the idea that Octospora and Anthracobia are closely related. Similarly, glycogen also appears before spores are enclosed by delimiting membranes. This feature seems very consistent in various groups of fungi during ascosporogeneis (Wells, 1972).

Before epispore formation, electron dense clumps were found in the perisporic sac of Octospora similar to those found in Aleuria and Anthracobia (Merkus, 1974) and in the species of Peziza (Merkus, 1975; Dyby and Kimbrough, 1987). This material is not interpreted as secondary wall or ornaments, because it disappears very soon after the

perisporic sac expands. It appears to contribute to the formation of the radial spinelike fibrils of the epispore. The precursors of the epispore layer in Octospora exhibit a tubular texture (Fig. 1.35) which is clearly shown in grazing sections. The diameter of these tubules is very regular and arranged in a fixed space. The cerebriform, mosaic feature on the epispore layer probably is due to the swelling of primary spore wall which results in the binding wall material cracking at the top of spinelike structure.

After primary wall formation in Pulvinula, the outer investing membrane expands to form a perisporic sac (Fig. 1.46). But differing from the previous two genera, the secondary wall material is not clearly detected in the perisporic sac (Fig. 1.38). Once the secondary wall material appears in the perisporic sac, it concentrates in a definite pattern only around the primary wall, leaving a loose fibrillar material throughout most of the perisporic sac. Thereafter, the epispore is differentiated from the concentrated wall material. This feature distinguishes spore ontogeny in Pulvinula from that of the other two genera.

The heterogeneity in the Aleurieae seen at the macroscopic and microscopic levels is also seen in this study on spore ontogeny and in previous ultrastructural investigations on septal structure (Kimbrough and Curry,

1986). It is apparent that the most significant events in the process of spore wall development take place within the perisporic sac. We have determined that spore ontogeny in Aleuria and Octospora is very similar to that of Mycolachnea, Trichophaea, and Scutellinia (Chapter II and III), while spore development of Pulvinula is similar to that found in Pezizaceae (Merkus, 1975; Dyby and Kimbrough, 1987), Helvellaceae (Gibson and Kimbrough, 1988a, 1988b; Kimbrough *et al.*, 1990), Geopyxis (Kimbrough and Gibson, 1990), and Tuberales (Kimbrough *et al.*, 1992; Janex-Favre and Parguey-Leduc, 1980, 1983, 1985, 1988; Janex-Favre *et al.*, 1988; Parguey-Leduc *et al.*, 1987, 1988; Parguey-Leduc *et al.*, 1990). Since previous studies on Pezizaceae (Dyby and Kimbrough, 1987), Helvellaceae (Gibson and Kimbrough, 1988a, 1988b), Ascobolaceae (Wu and Kimbrough, 1991a, 1991b) and other Humariaceae (unpublished data) have shown that the type of spore ontogeny is very consistent at the generic and familial levels, we feel that a redefinition of the Aleurieae and a rearrangement of the genera of Humariaceae should be undertaken (see Chapter VII).

Abbreviations used in the figures:

AP: apicule; AS: ascus
CMC: concentric membrane complex
DB: dense body
DM: dense material
DS: dense spherical body
EP: epispore
ER: endoplasmic reticulum
GL: glycogen
IM: investing membrane
L: lipid body
M: mitochondria
MG: mass globule
N: nucleus
P: paraphysis
PM: plasma membrane
PS: perisporic sac
PW: primary wall
S: translucent space
SDM: spore delimiting membranes
SER: smooth endoplasmic reticulum
SPB: spindle polar body
SW: secondary wall
T: epiplasmic tonoplast membrane
TZ: translucent zone
V: vacuole

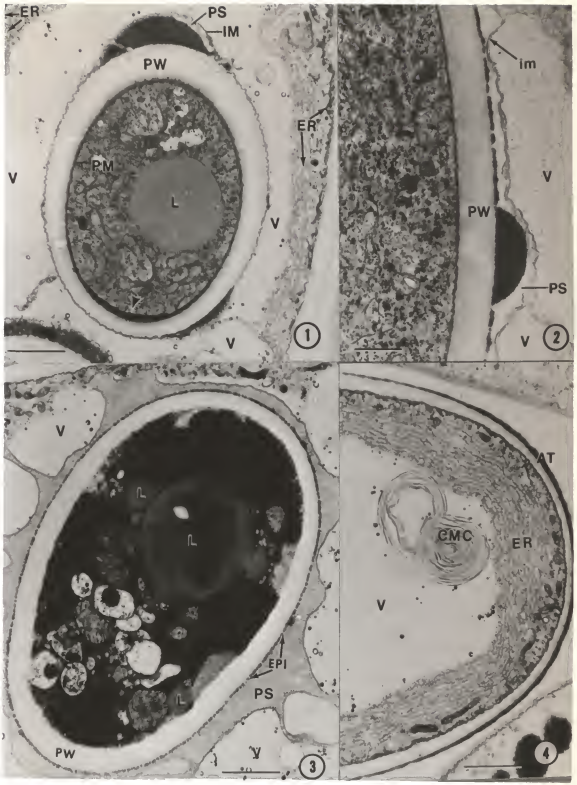
Figs. 1.1-1.4 Transmission electron micrographs of spore ontogeny in Aleuria aurantia.

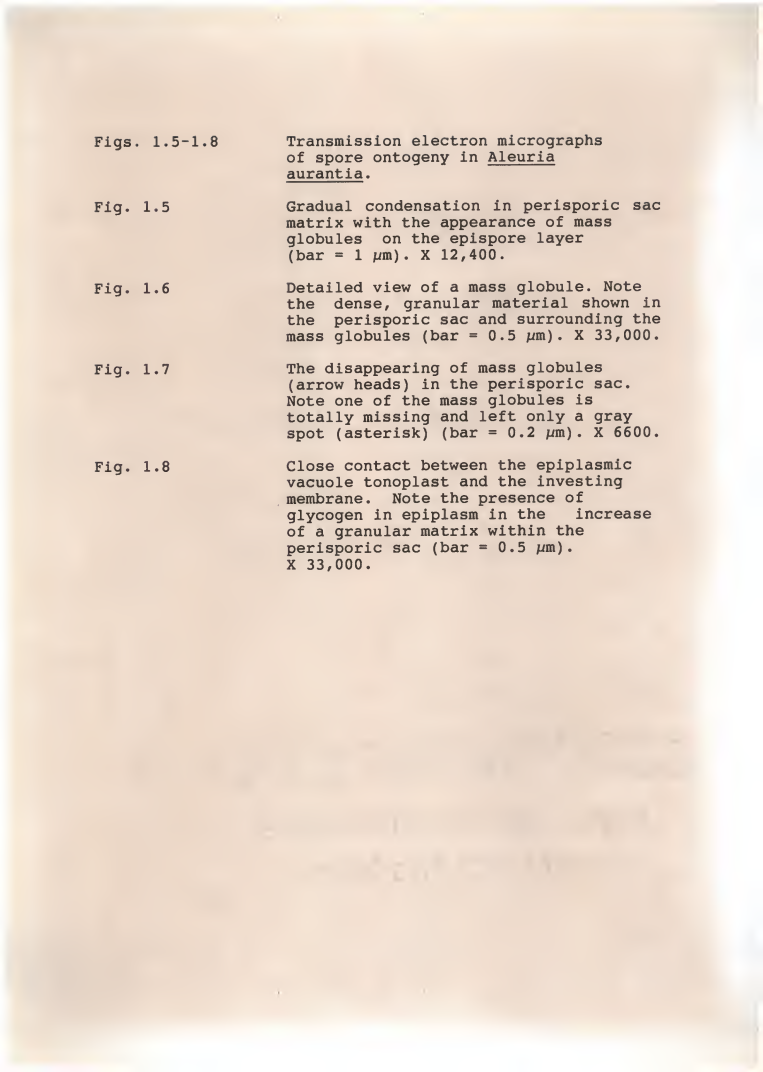
Fig. 1.1 Young ascospore showing dense secondary wall material within the perisporic sac which is bound by the outer investing membrane. Note another dense material deposited (arrow head) between the sporoplasm and primary wall and the large lipid droplet within. The epiplasm is highly vacuolate and the endoplasmic reticulum with dense contents is next to the ascal wall (bar = 1 μm). X 18,000.

Figure 1.2 Dense secondary wall material is deposited in the perisporic sac delimited by investing membrane and spreads along the primary wall (bar = 0.5 μm). X 30,000.

Figure 1.3 A young ascospore showing the perisporic sac and dotted precursors of an evenly distributed epispore layer (bar = 1 μm). X 18,000.

Figure 1.4 ER stacks, concentric membrane complex and the giant vacuole shown in the ascal tip area (bar = 1 μm). X 18,200.


ER
PS
IM
PW
ER
PM
V
L
V
V
1
im
V
PW
PS
V
2
V
L
L
L
EPI
PS
PW
3
AT
CMC
ER
V
4



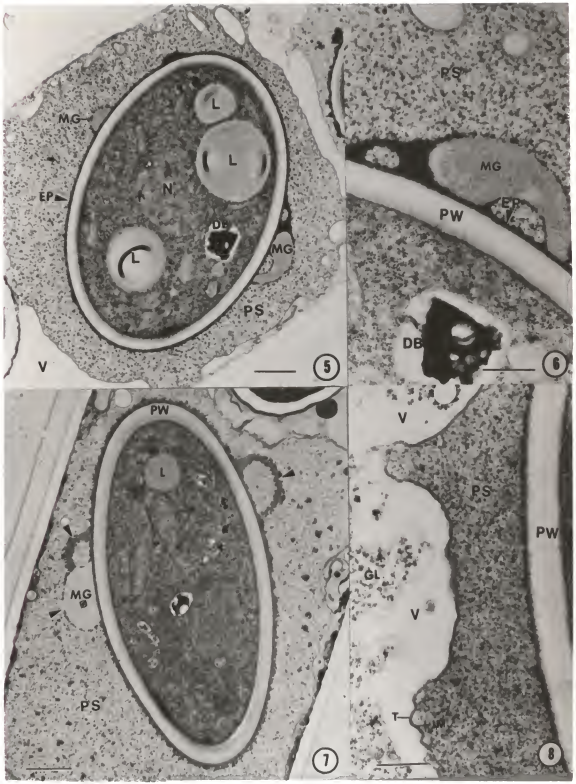
Figs. 1.5-1.8 Transmission electron micrographs of spore ontogeny in Aleuria aurantia.

Fig. 1.5 Gradual condensation in perisporic sac matrix with the appearance of mass globules on the epispore layer (bar = 1 μm). X 12,400.

Fig. 1.6 Detailed view of a mass globule. Note the dense, granular material shown in the perisporic sac and surrounding the mass globules (bar = 0.5 μm). X 33,000.

Fig. 1.7 The disappearing of mass globules (arrow heads) in the perisporic sac. Note one of the mass globules is totally missing and left only a gray spot (asterisk) (bar = 0.2 μm). X 6600.

Fig. 1.8 Close contact between the epiplasmic vacuole tonoplast and the investing membrane. Note the presence of glycogen in epiplasm in the increase of a granular matrix within the perisporic sac (bar = 0.5 μm). X 33,000.


MG
L
L
N
EP
DB
L
MG
PS
V
5
PS
MG
PW
EP
DB
6
PW
L
MG
PS
7
V
PS
PW
GL
V
T
8

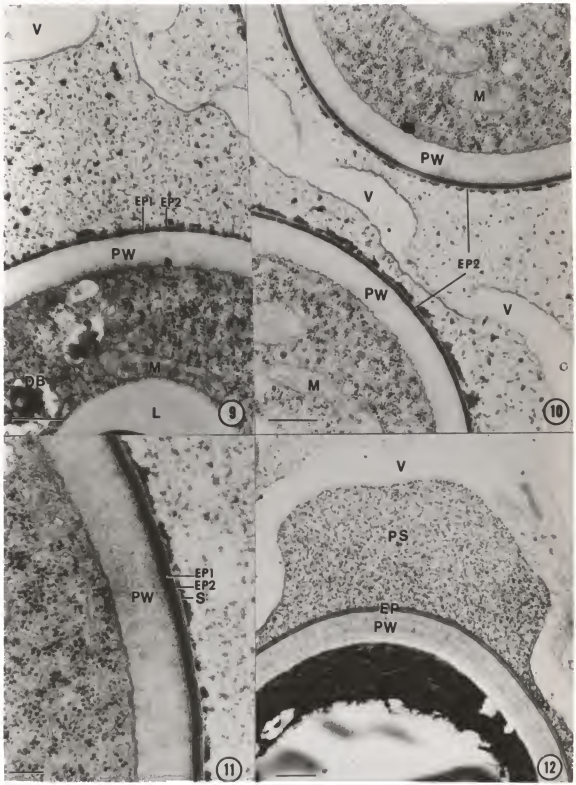
Figs. 1.9-1.12 Transmission electron micrographs of spore ontogeny in Aleuria aurantia.

Fig. 1.9 Formation of second layer of epispore. Note the dense wall material blocks suspended above the first layer of epispore (bar = 0.5 μm). X 33,000.

Fig. 1.10 The second epispore layer has already formed between dense blocks and first layer of epispore (bar = 0.5 μm). X 33,000.

Fig. 1.11 Detailed view of two epispore layers and a translucent space left above the second epispore layer (bar = 0.2 μm). X 57,000.

Fig. 1.12 Primary wall showing zonation surrounded by two epispore layers. Accumulation of material within the perisporic sac continues (bar = 0.5 μm). X 26,000.


V
EP1 EP2
PW
DB
M
L
9
M
PW
V
EP2
PW
V
M
10
PW
EP1
EP2
S
11
V
PS
EP
PW
12

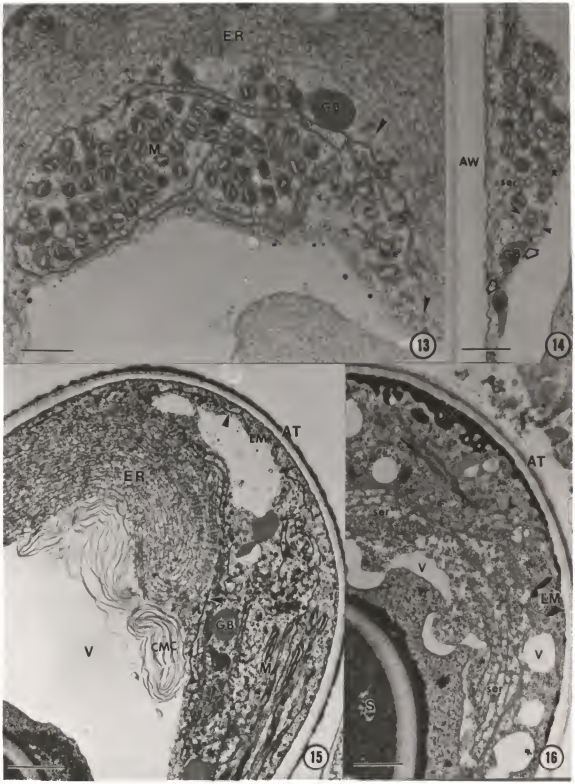
Figs. 1.13-1.16 Transmission electron micrographs of the ascal tip in Aleuria aurantia.

Fig. 1.13 Endoplasmic reticulum stack in the ascal tip area enclosing mitochondria and associated with a globular body. Arrow heads point out tiny vesicles next to ER and asterisks show the grazing section of ER (bar = 0.5 μm). X 34,000.

Fig. 1.14 The association of ER and mitochondria in the epiplasm. Note the distribution of microtubules in the periphery of SER and mitochondira (arrow heads). Arrows point out the connection of globular bodies with SER (bar = 0.5 μm). X 32,000.

Fig. 1.15 The distribution of organelles in the ascal tip showing the ER with dense inclusion (arrow heads) close to the margin of ER stack and ascal wall, and a number of globular bodies (bar = 1 μm). X 18,000.

Fig. 1.16 Lots of dense material released to the margin of the epiplasm next to ascal wall. Some less dense material is found around the ER (arrows) (bar = 1 μm). X 16,000.


ER
M
GB
AW
13
14
AT
EM
ER
V
CMF
GB
M
15
AT
V
EM
V
16

Figs. 1.17-1.20 Transmission electron micrographs of spore ontogeny in Aleuria aurantia.

Fig. 1.17 An ascus tip showing the distribution of ER above and ascospore ornament formation below. Inclusion contents within ER (arrows) differs with dense contents in ER next to ascal wall and in the lower portion of epiplasm associated with ascospores. Wall material in the perisporic sac condenses at two polar ends forming apiculi and dense spherical bodies (bar = 2 μm). X 7800.

Fig. 1.18 Detailed view of ascospore wall showing a well formed two layered epispore and an ephemeral translucent zone, separating dense wall blocks (ornaments) from the epispore, and dense spherical bodies within the perisporic sac (bar = 0.5 μm). X 26,000.

Fig. 1.19 Mature spore showing triangular secondary wall ornaments (bar = 1 μm). X 12,400.

Fig. 1.20 Detail view of mature spore wall showing homogenous nature of secondary wall material (bar = 0.5 μm). X 33,000.

ER
V
AP
PS
L
M
L
AP
17
LS
PS
AP
PW
TZ
EPI
L
18
SW
EPI
PW
20
SW
19

Figs. 1.21-1.25 Transmission electron micrographs of spore ontogeny in Aleuria aurantia.

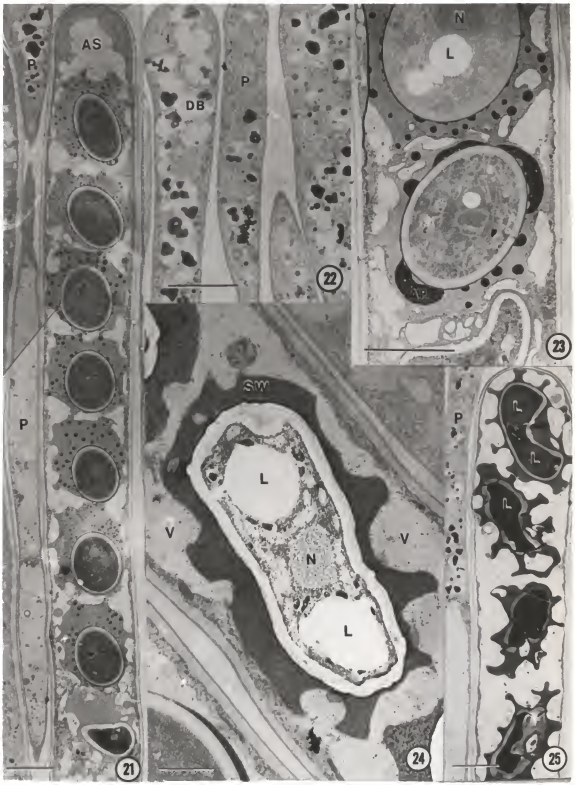
Fig. 1.21 Ascus with eight haploid ascospores. Note the gradual and more or less synchronous condensation undertaking within the perisporic sac of each (bar = 5 μm). X 3200.

Fig. 1.22 Paraphyses with numerous dense bodies (bar = 5 μm). X 4500.

Fig. 1.23 Hydrogen peroxide treated spore showing translucent lipid bodies and reticulate apicules (bar = 5 μm). X 5800.

Fig. 1.24 Hydrogen peroxide treated spore showing two typical lipid bodies removed and a single nucleus. Note the granular wall structure of secondary wall. Compared with Fig. 20 (bar = 2 μm). X 8750.

Fig. 1.25 Mature ascospores showing the reticulate ornaments and highly osmicated sporoplasm (bar = 5 μm). X 3100.


AS
P
DB
P
22
N
L
23
SW
L
V
N
V
L
24
P
25
21

Figs. 1.26-1.31 Transmission electron micrographs of spore ontogeny in Octospora euchroa.

Fig. 1.26 Spore delimitation in ascus with early formation of spore delimiting membrane (arrow heads). Meiotic division is underway. Note the spindle polar body (bar = 2 μm). X 9000.

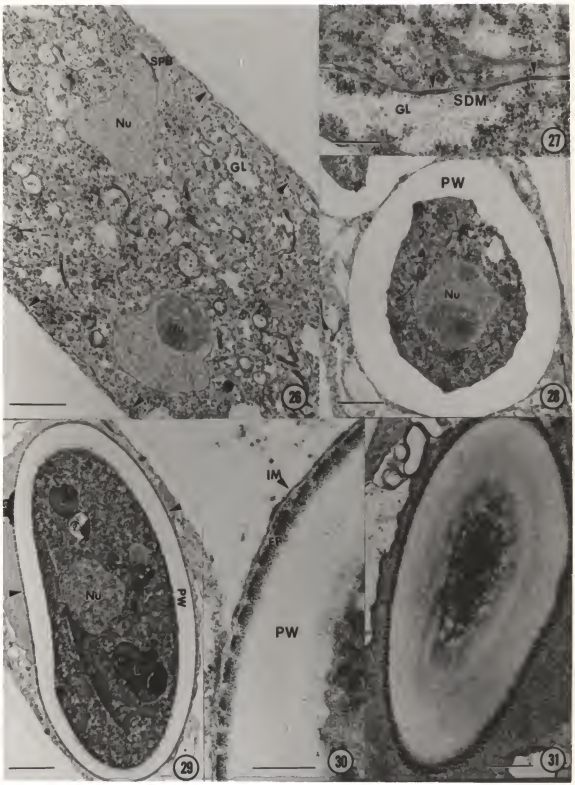
Fig. 1.27 Detailed view of SDM within which wall material appears to be deposited (arrow heads) and surrounded by glycogen (bar = 0.5 μm). X 32,000.

Fig. 1.28 Young ascospore with an electron translucent primary wall (bar = 2 μm). X 7000.

Fig. 1.29 Condensed material attached to epispore precursors (arrow heads) which appears as dashed lines (bar = 2 μm). X 7000.

Fig. 1.30 Detail view of spinelike precursors of epispore with a dense discontinuous line formed atop of these spinelike structures (bar = 0.5 μm). X 42,000.

Fig. 1.31 A grazing section of epispore layer showing "zebra-like" cracking feature (bar = 1 μm). X 14,000.


SPB
Nu
GL
26
GL
SDM
27
PW
Nu
28
Nu
PW
29
IM
PW
30
31

Figs. 1.32-1.36 Transmission electron micrographs of spore ontogeny in Octospora euchroa.

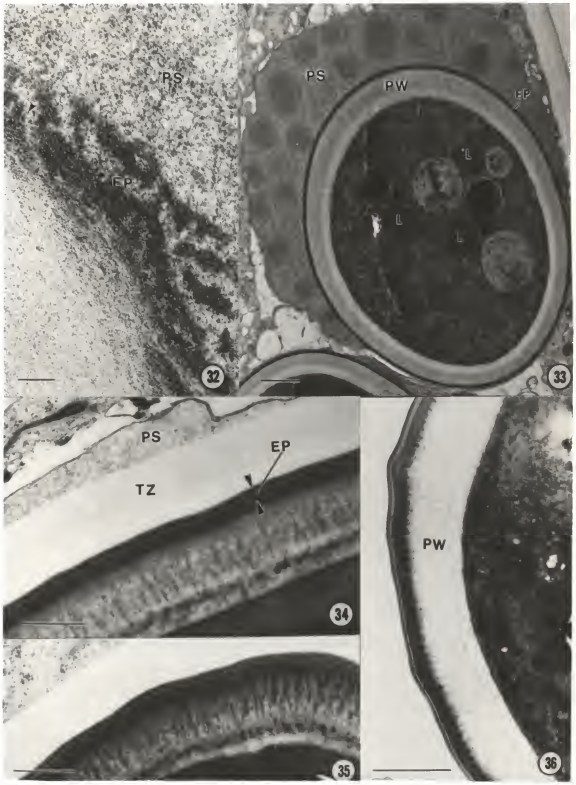
Fig. 1.32 Higher magnification of Fig. 31, showing the cross section of tubular spinelike precursors (arrow heads) of the epispore layer and its "zebra" feature on the surface (bar = 0.1 μm). X 120,000.

Fig. 1.33 Secondary wall material condenses into faintly stained spots in the perisporic sac (bar = 1 μm). X 12,000.

Fig. 1.34 Perisporic sac is separated from the epispore layer by a translucent zone (bar = 0.5 μm). X 40,000.

Fig. 1.35 Radiate, branched dense structures appear in the primary wall (bar = 0.5 μm). X 40,000.

Fig. 1.36 Mature ascospore showing a smooth outer secondary wall, translucent zone, two layered epispore with radial striations, and the thick, translucent primary wall (bar = 1 μm). X 25,000.


PS
EP
32
PS
PW
EP
33
PS
EP
TZ
34
35
PW
36

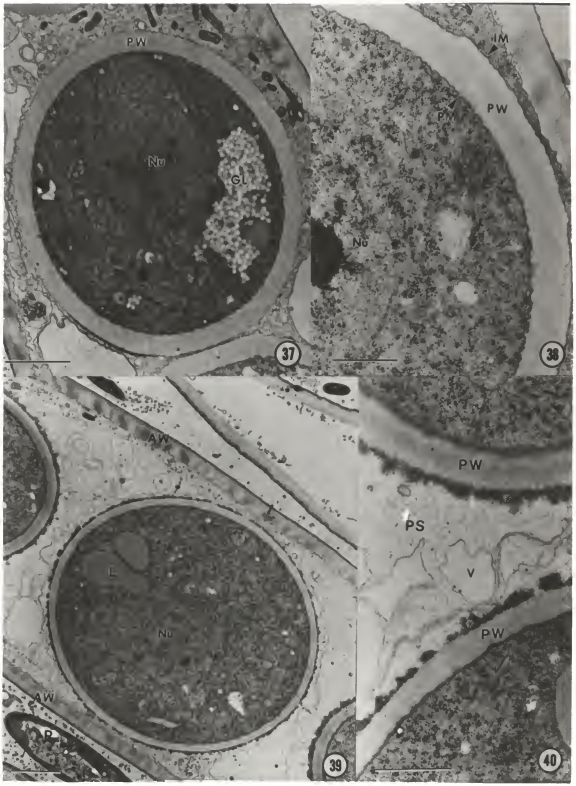
Figs. 1.37-1.40 Transmission electron micrographs of spore ontogeny in Pulvinula convexella.

Fig. 1.37. Young ascospore showing the primary wall and sporoplasm with glycogen and nucleus (bar = 2 μm). X 10,000.

Fig. 1.38. Primary wall of ascospore delimited by sporoplasma membrane and outer investing membrane (bar = 1 μm). X 20,000.

Fig. 1.39. Unique direct deposition of secondary wall material on primary wall with little distension of the perisporic sac (bar = 2 μm). X 7500.

Fig. 1.40. Detailed view of direct deposition of secondary wall material (asterisks) on primary wall and the fibrillar material in the outer perisporic sac (bar = 1 μm). X 24,000.


PW
Nu
GL
37
IM
PM
PW
Nu
38
AW
L
Nu
AW
P
39
PW
PS
V
PW
40

Figs. 1.41-1.45. Transmission electron micrographs of spore ontogeny in Pulvinula convexella.

Fig. 1.41. An evenly distributed epispore formed from the secondary wall material (aster) (bar = 2 μm). X 7500.

Fig. 1.42. Epispore is differentiated from the secondary wall material (asters) (bar = 1 μm). X 24,000.

Fig. 1.43. Detailed view of epispore layer showing radial and periclinal fibrils and residual wall material (asterisks) on EP (bar = 0.5 μm). X 39,000.

Fig. 1.44. Rodlets on mature spore surface as viewed in tangential section (bar = 0.5 μm). X 50,000.

Fig. 1.45. Cross section of mature ascospore wall showing minisculptured outer surface, a translucent zone, the epispore and the primary wall with an electron opaque outer zone and a translucent inner zone (bar = 0.5 μm). X 50,000.

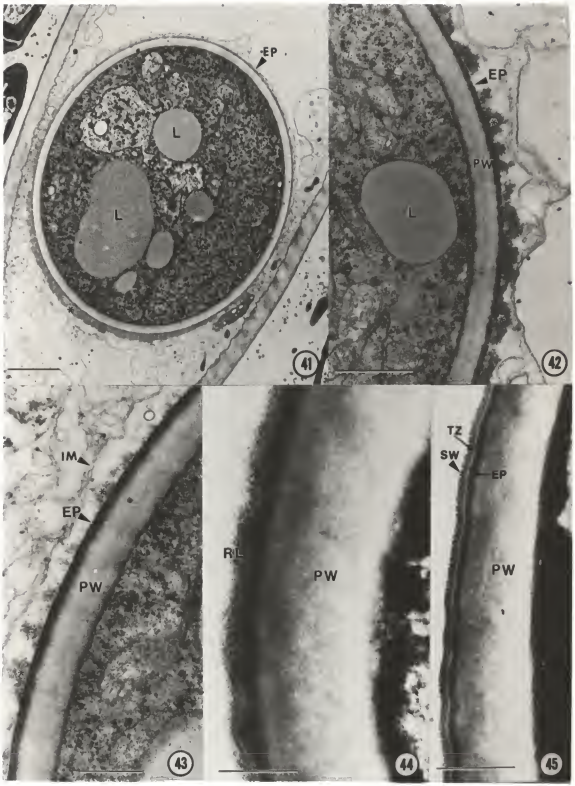

EP
L
L
41
EP
PW
L
42
IM
EP
PW
43
RL
PW
44
TZ
SW
EP
PW
45

Fig. 1.46. Comparison of spore wall development in Aleuria, Octospora and Pulvinula of the Aleurieae.

a. outer investing membrane.

b. inner sporoplasmic membrane.

c. perisporic sac.

d. primary wall.

e. condense clump.

f. precursors of epispore layer.

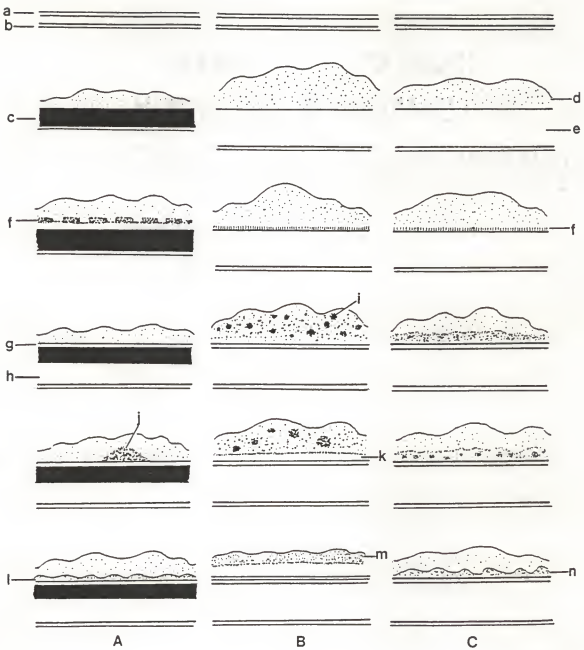
g. coagulated secondary wall material.

h. epispore layer.

i. mature ornaments, or secondary wall layer.

j. translucent zone.

k. radiate, branched structures.


a
b
c
d
e
f
f
g
h
i
j
k
l
m
n
A
B
C

CHAPTER II
ASCOSPOROGENESIS IN SELECTED GENERA OF CILIARIEAE

Introduction

The family Ciliarieae (as "Ciliariés") was initially proposed by Boudier (1885) to accommodate the genera of operculate Discomycetes which have excipular hairs and most apothecia with carotenoid pigmentation. It later became a tribe of the Humariaceae (Boudier, 1907). This circumscription was generally adopted by Le Gal (1947, 1953), Dennis (1978) and Rifai (1968), even though Hawksworth (1989) points out that the Humariaceae is an invalidly published name.

Eckblad (1968) placed members of Humariaceae in the Pyronemaceae and a new family Otideaceae. Even though he did not separate Pyronemaceae into subgroups, he proposed three phylogenetic lines trying to make some natural connections among the number of genera included. He treated Coprobia, Cheilymenia, Scutellinia, Humaria, Tricharina, Sphaerosporella, Sepultaria and Jafnea in the same line, based on the ascospore and excipular structures.

Arpin (1968) placed 11 genera, including the Ciliarieae, in the new family Aleuriaceae based mainly on the presence of carotenoid pigments. A similar

classification scheme was followed basically by Kimbrough (1970). Arpin classified this family into three groups based on the difference in the ratio of τ- to ß-carotene. He included Cheilymenia, Coprobia and Scutellinia together with Geopyxis in the first group with higher ratio of τ- to ß-carotene. However, Geopyxis has been shown by Gibson and Kimbrough (1990) to possess a type of spore ontogeny and septal structure similar to those of Helvella, even though Geopyxis produces uninucleate ascospores and has other characters unlike Helvella.

Recently, Korf (1972) erected a subfamily Scutellinioideae (Pyronemataceae) to include the fungi producing ascospores with or without guttules and mostly containing carotenoids in the apothecial tissue. To replace Ciliarieae, Korf selected the tribe Scutellinieae under which he placed three genera, *i.e.*, Cheilymenia, Coprobia, and Scutellinia. These genera have been chosen as the subjects in this study.

Rifai's (1968) system is temporarily followed in which he recognizes four tribes. The distinguishing features of his tribe Ciliarieae is in the combination of habitat, apothecial anatomy, and the type of hairs and ascospores. Most of the Ciliarieae are found on dead wood, dung, or other vegetable remains, rarely on ground which is the major habitat for the fungi in Aleurieae. Most of

Ciliarieae produce rooting hairs differentiated from the inner part of excipular tissue, although none of the species in Coprobia are characterized with this kind of excipular hairs. The inconspicuous, hyphoid superficial hairs, production of ascospores with rib-like, longitudinal striations and its exceptionally broad, apically inflated paraphyses make Coprobia a genus distinct from Cheilymenia (Denison 1964; Moravec 1988). However, after a reevaluation of these characters, Coprobia was recently treated as a synonym of Cheilymenia and placed under it as a section-Coprobiae (Moravec 1990).

According to Le Gal's (1947) studies at the light microscopic level on the formation and chemical composition of spore ornamentation, Scutellinia pseudotrechispora (Schroeter) Le Gal, Melastiza chateri (W. G. Smith) Boud., M. miniata (Fckl.) Boud., Aleuria aurantia (Pers. ex Hook.) Fckl., A. bicucullata Boud., A. luteonitens (Berk. & Br.) Gill. were treated as the type producing complex ornamentation. In this type, the ornaments originate partly within the vacuoles and partly from within the perispore. She thought that the globular masses inside the perisporic sac played an important role in the formation of ornaments, especially in the reticulate sculpturing. Globular masses have been found in A. aurantia at the ultrastructural level (Chapter II). While in the genus of Scutellinia, not all

species form ornaments as in S. pseudotrechispora, but actually most of them share the same type of ornaments as in Cheilymenia and Coprobia. They produce simple callose-pectic ornamentation which is stained by cotton blue and naphthylene blue.

Merkus (1974) did extensive ultrastructural studies on ascosporogenesis in the family Pyronemataceae, including species such as Cheilymenia pulcherrima (Crouan) Boud., S. armatospora Denison, and S. scutellata (L. ex Fr.) Lambotte. However, various stages of spore formation were not complete. Merkus (1974) compared spore formation in members of the Pyronemataceae with Ascodesmis microscopica (Crouan) Seaver and A. nigricans van Tiegh (Merkus, 1973), and thought that in Ascodesmis the secondary wall material builds up to form the final ornamentation pattern, while ornamentation patterns in the Pyronemataceae arise only through redistribution of secondary wall material that is already present. Later, Merkus (1976) studied members of most of the families of Pezizales (Morchellaceae, Helvellaceae, Rhizinaceae, Thelebolaceae, Sarcoscyphaceae and also part of the Pyronemataceae), including Coprobia granulata (Bull. ex Mérat) Boud. She outlined seven types of secondary wall formation, among which C. granulata was treated as an independent group because its secondary wall material condenses to form a complete ornamentation pattern

on the epispore that finally disappears as the ascospore matures. Nevertheless, she treated Cheilymenia and Scutellinia as having the same type of secondary wall formation as Aleuria.

Kimbrough and Curry (1986) studied septal structures in the apothecial tissues of species of Cheilymenia and Scutellinia and found that they all shared basically an identical septal pore plugging pattern, especially in the ascal base that is very similar to that found in Ascobolaceae. The integrity of Ciliarieae is also supported by the ultrastructural studies on ascal dehiscence (Brummelen, 1981; Samuelson, 1977). The genera all share the Octospora type of operculum. Since it has consistently been found that genera in the same family may possess the same type of spore ontogeny and septal structures (Curry and Kimbrough, 1983; Kimbrough and Curry, 1985; Kimbrough and Gibson, 1989; Dyby and Kimbrough, 1987; Wu and Kimbrough, 1991a; Gibson and Kimbrough, 1988a, 1988b; Kimbrough *et al.* 1990; Brummelen, 1989; unpublished data), we felt that a thorough investigation of ascospore formation in genera of Ciliarieae was needed to determine whether these data would correlate with other ultrastructural features. Furthermore, we hope these data may shed more light on the understanding of the systematic and phylogenetic relationships in the Humariaceae.

Material and Methods

Field collections were processed for TEM following the procedures described by Curry and Kimbrough (1983). The following specimens were observed: Cheilymenia coprinaria (Cke.) Boud., on cow dung, SW 20th Av., near I-75, Gainesville, Alachua Co,.Florida, collected by J. W. Kimbrough on Feburary 11, 1983 (FLAS-F53436); C. stercorea (Pers. : Fr.) Boud., on cow dung, SW 20th Av., near I-75, Gainesville, Alachua Co., Florida, collected by J. W. Kimbrough, on Feburary 11, 1983 (FLAS-F53435); Coprobia granulata (Bull. ex Mérat) Boud., on cow dung, Sugarfoot Hammock, on south side of SW 20th Av., approximate 0.5 mi., west of I-75, Gainesville, Alachua Co., Florida, collected by J. W. Kimbrough, on January 27, 1987 (FLAS-F55143); Scutellinia scutellata (L. ex Fr.) Lambotte, on wood stump covered with moss, behind Art Center, Highland Biological Station, Highland, North Carolina, collected by C.-G. Wu, on June 25, 1989 (FLAS-F55434); on soil-wood chips, moss yard/garden, at 154 NE 19Y,.Seattle, Washington, collected, by S. Ammirati, on June 5, 1989 (FLAS-F55433); in green house of Department Plant Pathology, University of Florida, Gainesville, Alachua Co., Florida, collected by D. Samuelson (unacessioned); S. pennsylvanica (Seav.) Denison, on dead wood, Ledges St. Park, Boone Co., Iowa, collected by A. Elliot, on June 25, 1983 (FLAS-F53630).

Plastic blocks were sectioned on an LKB Huxley ultramicrotome with a diamond knife. After poststaining with uranyl acetate and lead citrate, the sections were examined at 60 kv, on a JEOL 100-CX electron microscope.

Results

Cheilymenia stercorea

Before meiosis is complete, spore delimiting membranes (SDM) are already present beneath the ascal plasma membrane from which the SDMs originate (Figs. 2.1, 2.2). The fibrillar primary wall is formed between SDMs which enclose one nucleus, mitochondria and other organelles for each spore (Figs. 2.3, 2.4). The outer investing membrane of SDMs expands to form a perisporic sac in which secondary wall material is deposited (Figs. 2.5, 2.6). Numerous vacuoles of the epiplasm remain between the perisporic sacs of adjacent spores.

As condensation of secondary wall material begins, the density of the perisporic sac changes (Fig. 2.7). Before condensation (Fig. 2.6), the matrix of the perisporic sac is composed of evenly distributed granular material, but afterwards the wall material coagulates unevenly resulting in some areas with loose fibrils and others with mosaic dense clumps (Figs. 2.7-2.10). In this stage, very small lipid droplets are rarely found inside the sporoplasm, and

vacuoles of the epiplasm coalesce to separate the spores (Figs. 2.7, 2.8). Synchronously, radial spinelike epispore precursors appear to form from the secondary wall material and adhere to the surface of the primary wall (Figs. 2.8, 2.9). As condensation occurs, an electron-translucent space appears above the epispore layer (Fig. 2.9). The space is later occupied by secondary wall material forming the second layer of the epispore. A similar procedure is repeated a few times until the formation of a laminated epispore is complete (Fig. 2.10). Afterwards, an electron translucent zone is created by a redistribution of secondary wall material which congeals above the epispore layer and forms a dark band (Fig. 2.10). Within the perisporic sac some electron-dense clumps appear as reticulate structures (Figs. 2.10, 2.11) and some are globular or ellipsoid bodies (Fig. 2.10). These globular bodies contain very unique inclusions which are composed of electron-translucent rodlet structures embedded inside the dense matrix (Fig. 2.10). A translucent zone delimited by a dark band is more prominent when ascospores approach maturity. At this stage, the dark band becomes thicker (Fig. 2.12) and corresponding to this change, the edge of the perisoporic sac becomes electron-dense (Figs. 2.9-2.12). Epispore layers can be separated into two subgroups, i.e. an electron-dense, inner part next to the primary wall

and a less opaque, outer part next to the translucent zone (Figs. 2.11, 2.12). The epispore and primary wall in this stage have a periclinal zonation of microfibrils (Figs. 2.11, 2.12).

Mature ascospores are enclosed by spore sheaths which are formed by the complete separation of the secondary wall from the epispore (Figs 2.13, 2.14). At this stage, no obvious oil guttules are detected within the sporoplasm, instead, two large vacuoles are formed. Between the primary wall and sporoplasmic membrane, very dense clumps are deposited (Figs. 2.13, 2.14).

Cheilymenia coprinaria

Ascosporogenesis is exactly the same in C. coprinaria as in the previous species. Gradual condensation of secondary wall material also occurs in the perisporic sac (Figs. 2.15, 2.16). A translucent zone is also formed separating the epispore layer from a dense band which becomes thicker as spores mature (Fig. 2.16). The epispore layer includes numerous periclinal lines (Fig. 2.16) in which those next to the primary wall are more electron dense than the others (Figs. 2.15, 2.16). A mosaic pattern of unevenly distributed wall material is also found inside the perisporic sac due to the condensation of the wall matrix. Globular bodies are also found consistently in the

perisporic sac in this species. Their composition pattern is very similar to the former species with numerous electron-translucent rodlet structures embedded in an opaque matrix (Fig. 2.15). The primary wall in the mature ascospores also becomes zonate and the perisporic sac is highly reduced (Fig. 2.15). The dark band of secondary wall material in the perisporic sac becomes much thicker, the perisporic sac becomes thinner, and the outer wall totally detaches from epispore forming a spore sheath. Condensed bands may also be found by the margin of perisporic sac next to the epiplasmic vacuoles (Fig. 2.16). Although mature ascospores are smooth under the light microscope, they do show premature ornaments in the spore sheath at the ultrastructural level (Fig. 2.16, arrow heads).

Coprobia granulata

After meiosis, haploid nuclei are enclosed by spore delimiting membranes (Fig. 2.17), between which primary wall material is deposited (Fig. 2.19). The primary wall is usually fibrillar and electron-translucent (Fig. 2.20). Soon thereafter, the outer investing membrane is dilated to form a perisporic sac (Fig. 2.18) in which secondary wall material accumulates (Fig. 2.20). At this stage, the epiplasm is highly vacuolate and the sporoplasm has a few small lipid bodies. Through condensation of what appears to

be secondary wall material, epispore precursors are formed within the perisporic sac (Figs. 2.20-2.22). The epispore precursors are spinelike structures which are arranged on the surface of primary wall and coagulated into fragments (Fig. 2.21) which are later connected into a continuous layer (Fig. 2.22). Within the space between the investing membrane and tonoplast membrane, some dense material accumulates (Fig. 2.21, arrow heads).

Secondary wall material condenses randomly within the perisporic sac and the condensed granules are very small and barely detectable (Fig. 2.23). Only a few extremely small lipid droplets are present in the sporoplasm (Figs. 2.17, 2.20, 2.23). The gradual condensation is not as obvious as in the previous genus, but at higher magnification, unevenly distributed secondary wall material in the form of small condensed particles in the perisporic sac can still be distinguished (Figs. 2.24, 2.26, 2.29). Condensed wall material is very obvious on some areas of the epispore layer (Figs. 2.24, 2.25, arrow heads), usually leaving a space above the epispore layer where a second layer of epispore is formed later (Figs. 2.26, 2.28). Different structural images are observed on different planes of grazing sections of the epispore layer (Figs. 2.26, 2.27). Spinelike structures (Fig. 2.26, asterisk) are seen from one angle; however, in another view (Fig. 2.27) a

reticulate configuration is seen. When the synthesis of the epispore layer is complete, a translucent zone separates the secondary wall from the epispore layer (Fig. 2.28). The granular material on the epispore layer condenses into clumps (Fig. 2.28) and finally becomes the striated ornaments inside the perisporic sac (Fig. 2.27, arrow heads). When spores mature, the separation of a translucent zone from epispore layers may be enhanced and the perisporic sac including ornaments (asterisks), is reduced into a spore sheath (Fig. 2.30).

Scutellinia scutellata

In the juvenile stage of spore ontogeny, haploid nuclei are always enclosed by spore delimiting membranes (Fig. 2.31) between which an electron translucent primary wall is deposited (Fig. 2.32). During the early stages of spore ontogeny, the epiplasm is filled with mitochondria, small vacuoles, and numerous dense bodies. The outer investing membrane expands slightly to form a perisporic sac (Fig. 2.34). Since secondary wall material is tightly packed within the perisporic sac, young spores appear to be encircled by a dark band (Figs. 2.33, 2.34). Fibrillar bodies are usually found within the sporoplasm (Fig. 2.33). Before the outer investing membrane is highly dilated,

condensation within the perisporic sac has already begun (Fig. 2.35).

The first layer of epispore is formed on the primary wall by the condensation of secondary wall material in the form of radiating spinelike fibrils (Fig. 2.36). A granular matrix of electron-dense secondary wall material accumulates within the perisporic sac. When the condensed granules settle onto the epispore layer, a translucent line is created above the first layer of epispore (Fig. 2.37) and is then transformed into the second layer of epispore (Fig. 2.40). With continued deposition, up to four episporic layers will be formed (Fig. 2.41). Within the perisporic sac, an uneven condensation of wall matrix results in a mosaic pattern within the perisporic sac. Within the lightly opaque area, dense clumps of fibrils appear (Figs. 2.37, 2.39). In this stage, the ascospore is surrounded by numerous vacuoles (Fig. 2.38), and especially in the lightly opaque area of the perisporic sac, pitted depressions occur on the investing membrane (Fig. 2.38).

As spores mature, the sporoplasm contains numerous guttules and becomes highly osmophilic (Fig. 2.39), and the epispore has four distinct layers (Fig. 2.41). All of the condensed granules in the perisporic sac later gradually congeal into pulvinate clumps and leave a very clear background in the matrix (Fig. 2.42). At this stage,

vacuoles occur in the perisporic sac and later fuse with epiplasmic vacuoles (Fig. 2.42). Accompanying this vacuolation, the perisporic matrix, excluding the clumps, finally disintegrates, leaving only an undulated secondary wall (ornaments) on the spore surface (Figs. 2.43, 2.44). Translucent bands are consistently found in the final stages of spore ontogeny at the base of the ornaments (Figs. 2.42, arrow heads, 2.44). The epiplasm at this stage is completely vacuolate.

Scutellinia pennsylvanica

An electron translucent primary wall forms between the outer and inner spore delimiting membranes (Fig. 2.45). The young spore is uninucleate and contains conspicuous fibrillar bodies, mitochondria, but no lipid (Fig. 2.46). The outer investing membrane expands, forming a dilated perisporic sac (Figs. 2.46, 2.47). There is already an accumulation of granular, electron opaque secondary wall material within the perisporic sac (Figs. 2.46, 2.47). The appearance of fibrillar bodies precedes the appearance of lipid bodies (compare Figures 2.46 and 2.48). Later on, condensation of secondary wall material takes place in the perisporic sac and an epispore layer is formed first on the primary wall (Figs. 2.48-2.50). The epispore is composed of four distinguishable layers (Figs. 2.52-2.54). Simultaneous

to the formation of the epispore, secondary wall material in the perisporic sac becomes unevenly distributed with broad radiated spikes separating by more electron dense areas (Figs. 2.48, 2.49). In this stage, one or few small sporoplasmic lipid droplets are found and the spore is surrounded by a few vacuoles (Fig. 2.47) that later coalesce to separate the spores (Fig. 2.48).

Accompanying the deposition of secondary wall material, more condensation occurs in the perisporic sac (Fig. 2.50). Secondary wall material congeals into mounds of fibrillar structures (Fig. 2.50, arrow heads; Figs. 2.52-2.54). The less dense broad radiated spikes are at this stage transformed into globules to which vacuoles in the epiplasm are frequently found associated (Fig. 2.51, asterisks). These globulular structures are less electron opaque and contain very fine filaments (Fig. 2.52). In the condensed area of the perisporic sac, cerebriform "tubular" structures are always found (Figs. 2.52-2.54). Vacuolation occurs in the less opaque region within the perisporic sac (Fig. 2.52, asterisks). These vacuoles will fuse later with the epiplasmic vacuoles and create pitted depressions on the investing membrane outlining the ornaments. Translucent bands similar to those found in S. scutellata are usually found at the base of ornaments (Figs. 2.52, 2.54). The granular matrix around the ornaments (Fig. 2.54) eventually

condenses onto the ornament and the perisporic sac membrane collapses onto the spore wall (Fig. 2.53).

Discussion

Two species of Scutellinia studied have exactly the same type of spore ontogeny. These similarities include gradual condensation of secondary wall material, Aleuria-type of epispore formation (Wu and Kimbrough 1991b), fibrillar bodies in the sporoplasm, uneven electron density in the perisporic sac (*i.e.*, some areas are less opaque and globular, and some are electron dense and angular), vacuolation in the perisporic sac, and the presence of translucent bands at the base of ornaments. The less opaque, globular area in the perisporic sac (Figs. 2.38, 2.39, 2.51) is probably the "masses globuleuses périsporiques" as described by Le Gal (1947) in Scutellinia pseudotrechispora, since she found that dense wall material was only detected around these structures. This phenomenon is exactly the same as was observed in the current ultrastructural study. The only difference between the less opaque, globular areas and Le Gal's "masses globuleuses périsporiques" is their origin. The former is caused by an uneven distribution of secondary wall material within the perisporic sac; however, the latter was thought by her to be incorporated from the epiplasm.

In the two species of Cheilymenia studied, an identical pattern of ascosporogenesis is shown, including their gradual condensation of secondary wall material, mosaic type of wall material distribution, Aleuria-type of epispore formation, fibrillar bodies and limited number of lipid droplets in the sporoplasm, the appearance of globular bodies in the perisporic sac, the presence of translucent zone, and differentiation of a spore sheath. The production of eguttulate ascospores and differentiation of a spore sheath in the species of Cheilymenia and Coprobia are considered to be the most important characters separating them from other genera (Denison, 1959, 1964; Rifai, 1968; Korf, 1973). However, it seems difficult to dispute the fact that lipid droplets are indeed present at the EM level, even though they are very small and scarce.

Coprobia has basically the same type of spore ontogeny as Cheilymenia and Scutellinia, but with some minor differences such as the absence of fibrillar bodies in the sporoplasm and less pronounced condensation of secondary wall material in the perisporic sac. We do not agree with Merkus's (1976) conclusion that the final ornaments on the epispore disappear when ascospores mature. Based on this point, Merkus (1976) separated Coprobia from Cheilymenia and Scutellinia as possessing an independent pattern of secondary wall formation. We found that the final striated

ornaments are actually detached from epispore and became a part of spore sheath (Fig. 2.30). This phenomenon is exactly the same as found in the Cheilymenia (Fig. 2.16). In both genera the spore sheath may have a series of cyanophilic folds or blunt ridges. Besides the similarity of ascosporogenesis, septal structures of Coprobia are also the same as those of Scutellinia and Cheilymenia (unpublished data).

In the two species of Scutellinia studied, a spore sheath did not develop as in Coprobia and Cheilymenia, because the former lacks a translucent zone which allows for the differentiation of a loose spore sheath. There are still some translucent bands left at the base of ornaments which may be the traces of a translucent zone. Because the translucent zone is very obvious in the coprophilous fungi (Wells, 1972; Wu and Kimbrough, 1991a; Mims *et al.*, 1990; Brummelen, 1989), Scutellinia is possibly closely related to coprophilous genera. Most of the species of Scutellinia are found growing on dead wood or on the ground among mosses. Perhaps as taxa adapt to a coprophilous environment, taxa such as Cheilymenia develop a translucent zone and detachable secondary wall like Ascobolus. However, two sections of Scutellinia, Minutae Svr. and Pseudocheilymenia Svr., do produce a separable spore sheath. Species of these sections are distinct from

Cheilymenia by producing many guttules in the sporoplasm and without the yellow refractive color in mature ascospores when stained by cotton blue in lactic acid (Moravec, 1988, 1990; Schumacher, 1979).

Basically, all of the spore ornaments in these three genera are created by the redistribution of secondary wall material in the perisporic sac. This feature is supported from the evidence that when the condensed granules appear, some parts of the perisporic sac, especially at the marginal area, become fibrillar and less opaque. This conclusion agrees basically with Merkus's (1974) data. However, this redistribution phenomenon does not begin early, but only when the secondary wall material is fully deposited. Actually, redistribution and deposition of secondary wall material may proceed simultaneously. For instance, in S. pennsylvanica, the redistribution of wall material takes place just when the perisporic sac starts to expand (Fig. 2.49).

The number of oil drops or other guttules per ascospore seems to be constant in most species and was used by Boudier (1885, 1907) to distinguish taxa. This character was also used to help separate Cheilymenia and Coprobia from Scutellinia at the light microscopic level (Rifai, 1968; Korf, 1973; Moravec, 1990). Nevertheless, most of the "eguttulate" species of the Ciliarieae we studied all

actually produce oil droplets (Wu and Kimbrough, 1991a; unpublished data), even though these guttules are very small and of a limited number. This is perhaps the reason why they are not detectable with the light microscope.

The significance of fibrillar bodies within the sporoplasm of Scutellinia and Cheilymenia is unknown. However, it seems to be related to the spore wall formation, since they are only observed at the early stages of spore ontogeny. Similar structures have been found in Ascobolus (Wu and Kimbrough, 1991a, 1991b) and Mycolachnea (Chapter III).

If we compare all of the characters from the three genera in this study, they all share strong similarities and also, many of the features overlap between the genera (Fig. 2.55). This indicates that Ciliarieae is a very natural group and the genera are phylogenetically related.

CHAPTER II

Abbreviations used in the figures:

AS:	ascus
AW:	ascal wall
C:	condensed area
DB:	dense band
EP:	epispore
FB:	fibrillar body
G:	condensed granule
GB:	globular body
IM:	investing membrane
L:	lipid droplet
M:	mitochondria
Nu:	nucleus
O:	ornament
P:	paraphysis
PS:	persiporic sac
PW:	primary wall
SDM:	spore delimiting membrane
SM:	plasma membrane
SS:	spore sheath
TB:	translucent band
TM:	tonoplast membrane
TZ:	translucent zone
V:	vacuole

Figs. 2.1-2.6 Transmission electron micrographs of spore ontogeny in Cheilymenia stercorea.

Fig. 2.1 Haploid nuclei and spore delimiting membranes in ascus (bar=1 μm). X 16,000.

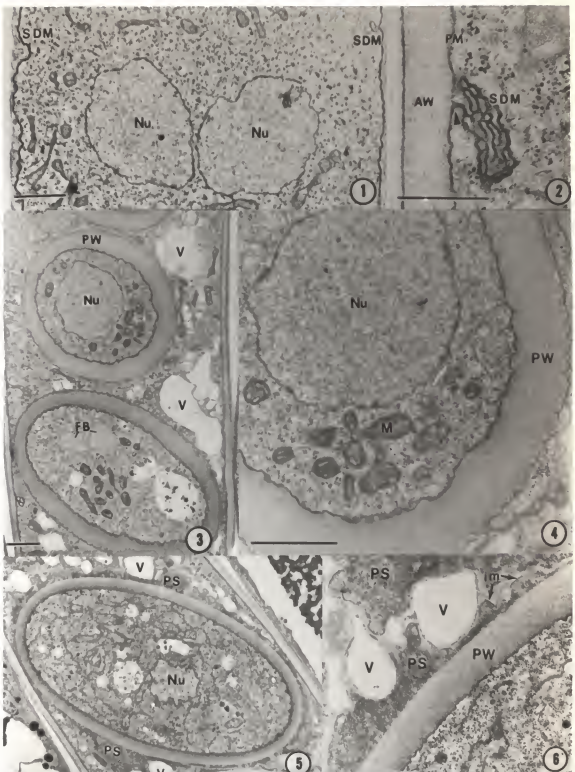
Fig. 2.2 Origin of spore delimiting membrane from sporoplasma membrane (bar=0.5 μm). X 57,000.

Fig. 2.3 Young ascospores with electron translucent primary wall (bar=1 μm). X 10,000.

Fig. 2.4 Detailed view of a young ascospore, showing its fibrillar primary wall and spore contents (bar=1 μm). X 26,000.

Fig. 2.5 A young ascospore with its dilated perisporic sac (bar=1 μm). X 10,000.

Fig. 2.6 Detailed view of the perisporic sac, within which secondary wall material is deposited (bar=1 μm). X 26,000.


SDM
SDM
Nu
Nu
1
PM
AW
SDM
2
PW
V
Nu
V
FB
3
Nu
PW
M
4
V
PS
Nu
PS
V
5
PS
m
V
V
PS
PW
6

Figs 2.7-2.12 Transmission electron micrograph of spore ontogeny in Cheilymenia stercorea.

Fig. 2.7 Highly expanded perisporic sac with an uneven distribution of secondary wall material, some areas showing only pale, fibrillar material (hollow arrow), and others very dense clumps (solid arrow heads) (bar=1 μm). X 10,000.

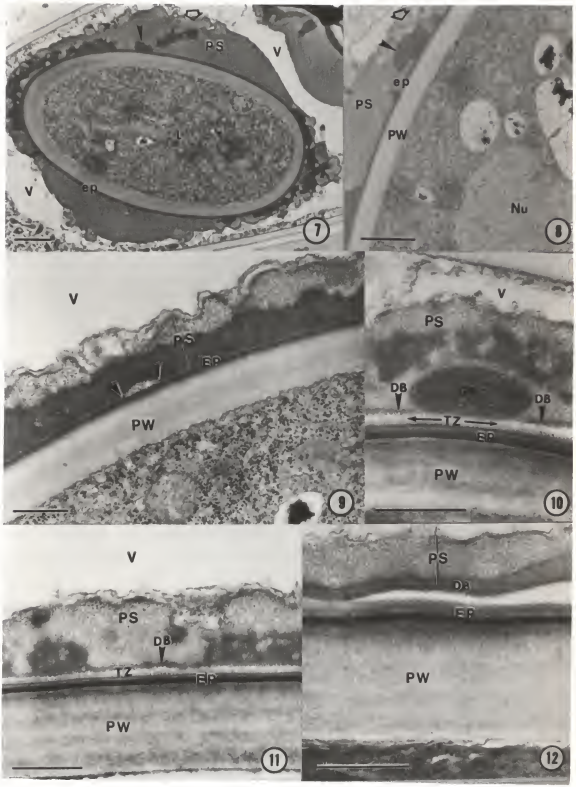
Fig. 2.8 Detailed view of the perisporic sac and spore wall structure in a young ascospore stage with an uneven distribution of secondary wall material in which some parts have a pale, fibrillar character (hollow arrow), and some with dense clumps (arrow head) (bar=1 μm). X 16,000.

Fig. 2.9 Dense clumps of granular material coagulates above the epispore layer leaving loosely fibrillar material in the outer zone of the perisporic sac next to epiplasmic vacuole. A thin translucent zone (arrow heads) forms djacent to the epispore layer (bar=0.5 μm). X 33,000.

Fig. 2.10 The translucent zone broadens to separate the epispore layer from the perisporic sac. At the same time, a dark band is formed next to the translucent zone and reticulate dense areas and globular bodies appear (bar=0.5 μm). X 57,000.

Fig. 2.11 Gradual condensation of wall material within the perisporic sac (bar=0.5 μm). X 44,000.

Fig. 2.12 Laminated epispore layer and greatly thickened dark band in the perisporic sac (bar=0.5 μm). X 57,000.


PS
V
ep
V
7
ep
PS
PW
Nu
8
V
PS
EP
PW
9
V
PS
DB
DB
TZ
EP
PW
10
V
PS
DB
TZ
EP
PW
11
PS
DB
EP
PW
12

Figs. 2.13-2.14 Transmission electron micrographs of spore ontogeny in Cheilymenia stercorea.

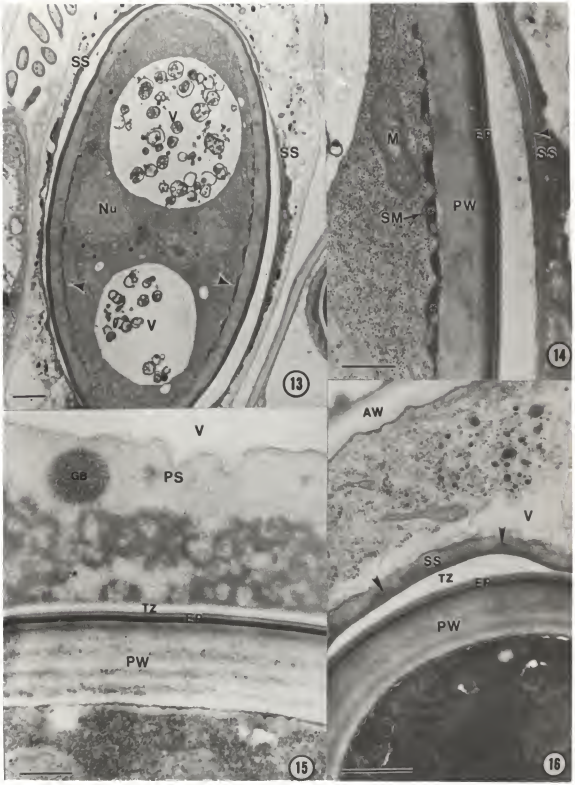
Fig. 2.13 Mature ascospore surrounded by a spore sheath detached from the epispore, the nucleus and two large vacuoles within, and the deposition of dense wall material in the inner side of primary wall (bar=1 μm). X 10,000.

Fig. 2.14 Detailed view of the wall deposition between sporoplasma membrane and primary wall, and the laminated secondary wall of the inner spore sheath (bar=0.5 μm). X 33,000.

Figs 2.15-2.16 Transmission electron micrographs of spore ontogeny in Cheilymenia coprinaria.

Fig. 2.15 Gradual condensation in the perisporic sac showing reticulate, dense structures, a globular body, and the presence of a translucent zone above the epispore layer (bar=0.5 μm). X 33,000.

Fig. 2.16 Spore section showing a zonate primary wall, darkly stained epispore, translucent zone and the secondary wall (arrow heads) still distinguishable in the spore sheath (or perisporic sac) (bar=0.5 μm). X 44,000.


SS
V
SS
Nu
V
13
M
EP
SS
SM
PW
14
V
GB
PS
TZ
EP
PW
15
AW
V
SS
TZ
EP
PW
16

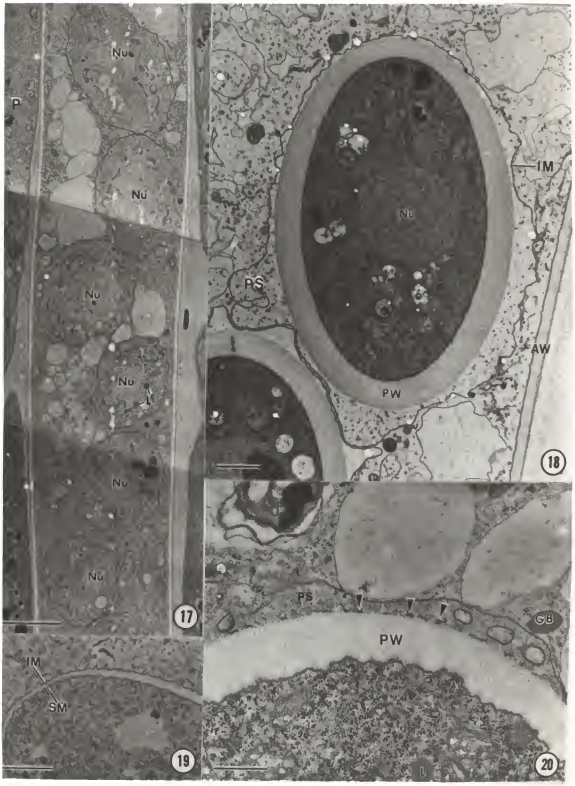
Figs. 2.17-2.20 Transmission electron micrographs of spore ontogeny in Coprobia granulata.

Fig. 2.17 An ascus showing very young ascospores enclosed only by spore delimiting membranes (bar=4 μm). X 5,000.

Fig. 2.18 A young ascospore with a thickened primary wall and a dilated perisporic sac (bar=1 μm). X 13,000.

Fig. 2.19 The primary wall of young ascospore delimited by an outer investing membrane and an inner sporoplasma membrane (bar=1 μm). X 16,500.

Fig. 2.20 Detailed view of an electron translucent primary wall and dense fragments (arrow heads) next to the primary wall due to the condensation of secondary wall material in the perisporic sac (bar=0.5 μm). X 32,000.


Nuc
P
Nu
Nu
Nu
Nu
Nu
Nu
17
IM
SM
19
P
IM
Nu
PS
AW
PW
18
PS
CB
PW
20

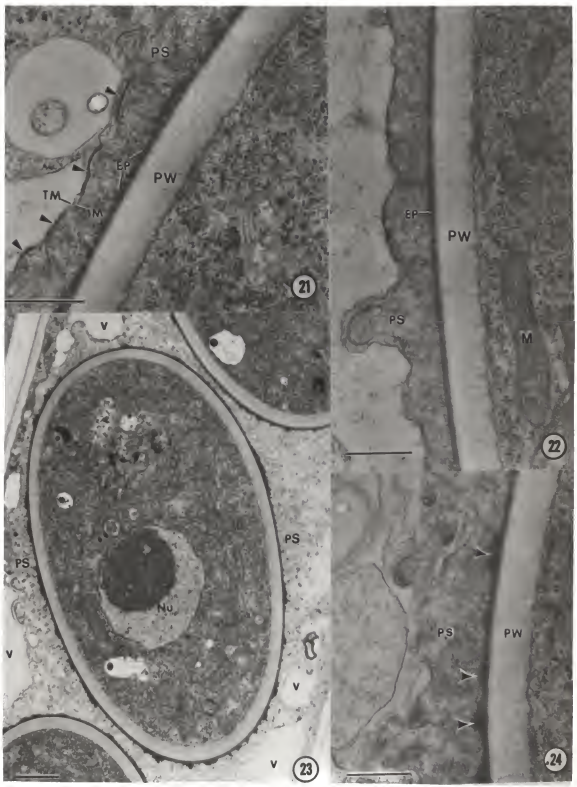
Figs. 2.21-2.24 Transmission electron micrographs of spore ontogeny in Coprobia granulata.

Fig. 2.21 Fragments of epispore precursors are formed on the primary wall and wall deposition (arrow heads) between the tonoplast membrane and outer, investing membrane (bar=0.5 μm). X 51,000.

Fig. 2.22 A layer of epispore with periclinal short radial microfibrils is formed on the surface of primary wall (bar=0.5 μm). X 40,000.

Fig. 2.23 An uninucleate ascospore showing the highly expanded perisporic sac and irregularily deposited secondary wall material (bar=1 μm). X 13,000.

Fig. 2.24 Some condensed granules (arrow heads) are coagulated adjacent to the epispore layer as other dense clumps appear to be entering the periphery of the perisporic sac (bar=0.5 μm). X 40,000.


PS
EP
PW
TM
M
21
EP
PW
PS
M
22
V
PS
PS
Nu
V
V
V
23
PS
PW
24

Figs. 2.25-2.30 Transmission electron micrographs of spore ontogeny in Coprobia granulata.

Fig. 2.25 Condensed blocks (arrow heads) are formed above an epispore layer (bar=0.5 μm). X 33,000.

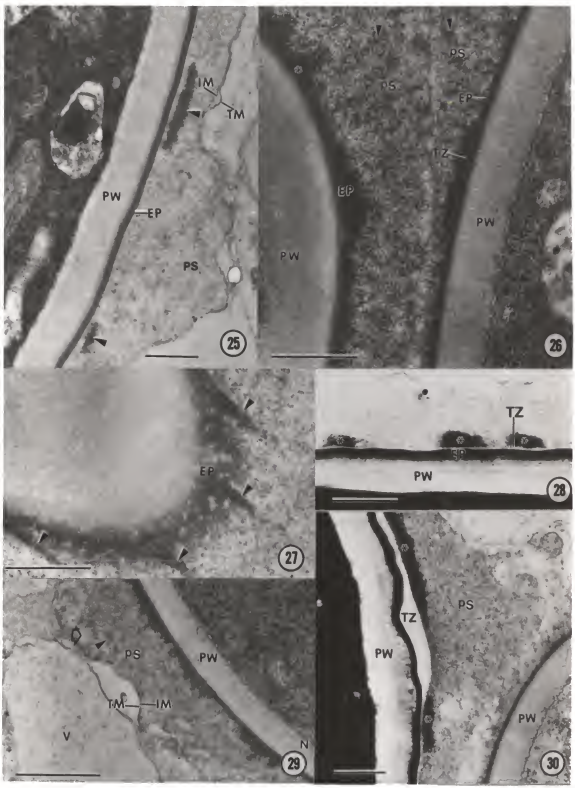
Fig. 2.26 Comparison of epispore wall structure in different sectioning planes. In the grazing section, an epispore wall shows filamentous structures (asterisk) and in the longitudinal section, spinelike precursors are connected laterally forming a smooth layer. Note the small dense granules clustered in the perisporic sac (arrow heads) (bar=0.5 μm). X 52,000.

Fig. 2.27 Reticulate feature of an epispore layer in a grazing section. Note the striated clumps (arrow heads) associated with the epispore (bar=0.5 μm). X 52,000.

Fig. 2.28 Wall layers and small ornaments (asterisks) of a mature ascospore (bar=0.5 μm). X 40,000.

Fig. 2.29 By the margin of perisporic sac, uneven condensation of secondary wall material in the perisporic sac results in some areas that become very pale (hollow arrow) and some areas become dense (arrow head) (bar=1 μm). X 26,000.

Fig. 2.30 Separation of the perisporic sac from a mature ascospore leaving a prominent translucent zone and pulvinate areas of secondary wall left behind in the spore sheath (bar=0.5 μm). X 32,000.


IM
TM
PW
EP
PS
25
PS
EP
TZ
EP
PW
PW
26
EP
27
TZ
PW
28
PS
PW
TM
IM
V
N
29
TZ
PS
PW
PW
30

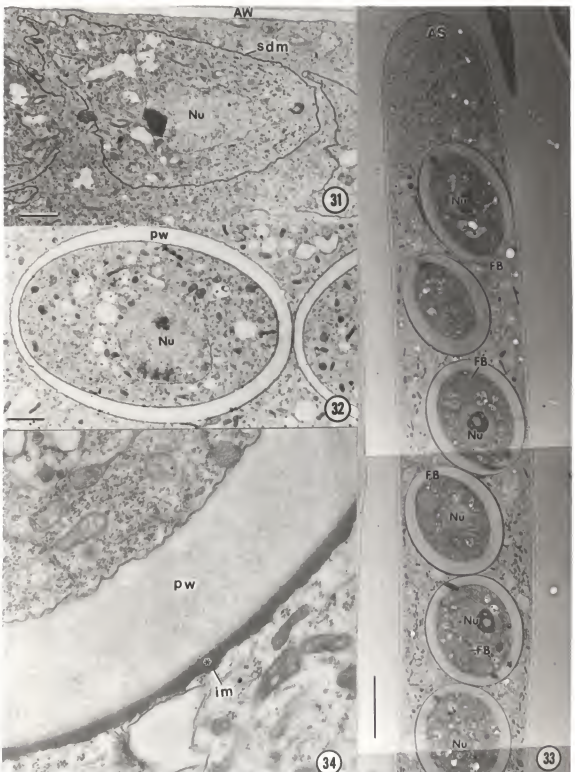
Figs. 2.31-2.34 Transmission electron micrographs of spore ontogeny in Scutellinia scutellata.

Fig. 2.31 Early stage of spore formation showing a haploid nucleus enclosed by spore delimiting membrane (bar=1 μm). X 11,000.

Fig. 2.32 A young uninucleate ascospore with an electron translucent primary wall (bar=1 μm). X 11,000.

Fig. 2.33 A young ascus with synchronously developing young ascospores (bar=5 μm). X 3,800.

Fig. 2.34 Detailed view of a young ascospore, with a primary wall and a slightly expanded perisporic sac (asterisk) with dense epispore wall precursors delimited by an investing membrane (bar=0.5 μm). X 38,000.


AW
sdm
Nu
31
pw
Nu
32
pw
im
34
AS
Nu
FB
FB
Nu
FB
Nu
Nu
FB
Nu
33

Figs. 2.35-2.41 Transmission electron micrographs of spore ontogeny of Scutellinia scutellata.

Fig. 2.35 An uneven distribution of wall material is shown in the perisporic sac in which some areas are pale (hollow arrow), and others are very dense (arrow head) (bar=1 μm). X 25,000.

Fig. 2.36 Formation of epispore in the perisporic sac (bar=0.5 μm). X 35,000.

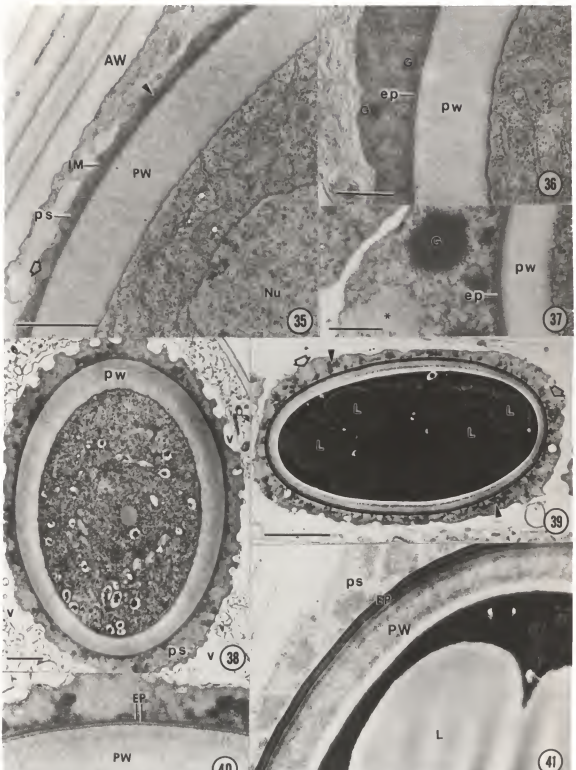
Fig. 2.37 Further condensation of wall material within the perisporic sac where some parts are pale (asterisk) and some are electron dense (bar=0.5 μm). X 32,000.

Fig. 2.38 An ascospore with the perisporic sac surrounded by vacuoles (bar=1 μm). X 11,000.

Fig. 2.39 An ascospore with a few sporoplasmic lipid droplets and condensation of secondary wall material within the perisporic sac. Globular and less opaque (hollow arrows) and electron dense and irregularly shaped (arrow heads) areas are noted (bar=4 μm). X 5,000.

Fig. 2.40 Two epispore layers are formed on the primary wall (bar=0.5 μm). X 30,000.

Fig. 2.41 Laminated epispore layers and its perisporic sac with mosaic pattern (bar=1 μm). X 27,000.

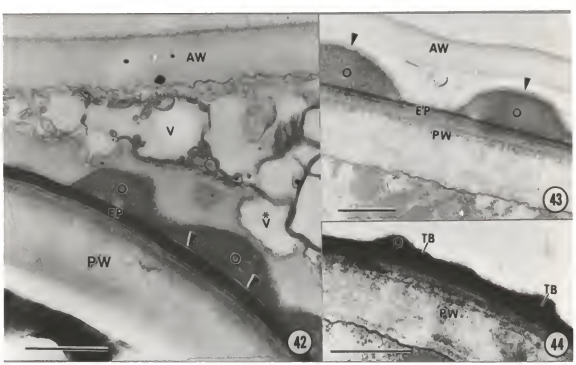

AW
IM
PW
ps
Nu
35
G
ep
pw
36
G
pw
ep
37
pw
v
v
ps
v
38
L
39
ps
EP
PW
L
41
EP
PW
40

Figs. 2.42-2.44 Transmission electron micrographs of spore ontogeny in Scutellinia scutellata.

Fig. 2.42 Ornament formation in the perisporic sac showing the presence of translucent bands (arrow heads) at the base of ornaments and vacuoles (asterisk) in the perisporic sac (bar=0.5 μm). X 50,000.

Fig. 2.43 The remains of the secondary wall material (arrow heads) coating the ornaments after the vacuolation in the perisporic sac (bar=0.5 μm). X 36,000.

Fig. 2.44 Ornaments of a mature ascospore showing the presence of translucent bands at their bases (bar=0.5 μm). X 50,000.


AW
V
EP
V
PW
42
AW
EP
PW
43
TB
TB
PW
44

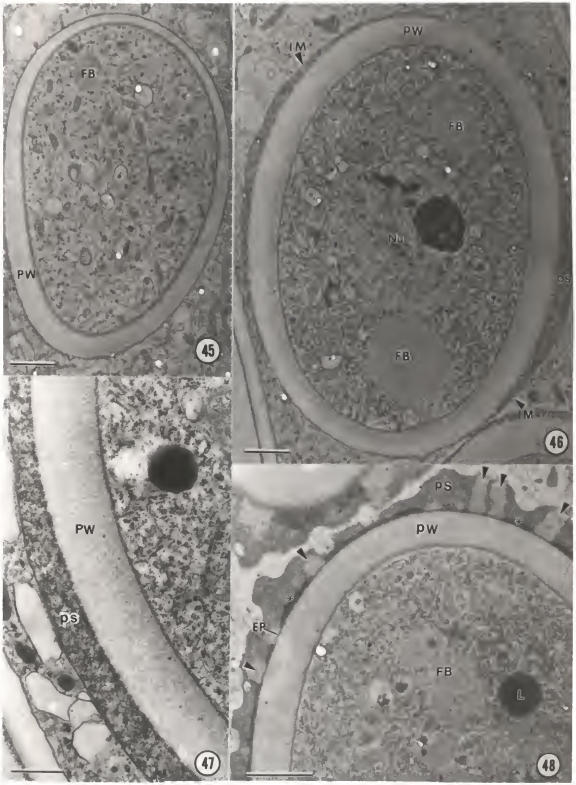
Figs. 2.45-2.48 Transmission electron micrographs of spore ontogeny in Scutellinia pennsylvanica.

Fig. 2.45 Early primary wall development in a young ascospore (bar=1.μm). X 13,000.

Fig. 2.46 A young ascospore with a slightly expanding perisporic sac. Note the presence of fibrillar bodies in the sporoplasm (bar=1 μm). X 13,000.

Fig. 2.47 Detailed view of the perisporic sac within which some secondary wall material is already deposited (bar=0.5 μm). X 32,000.

Fig. 2.48 A redistribution of secondary wall material in the perisporic sac results in some less opaque spikes (arrow heads) intermixed with electron dense bands. Note the presence of small lipid droplet and fibrillar body in the sporoplasm (bar=1 μm). X 20,000.


FB
PW
45
IM
PW
FB
NM
FB
IM
46
PW
PS
47
PS
PW
ER
FB
L
48

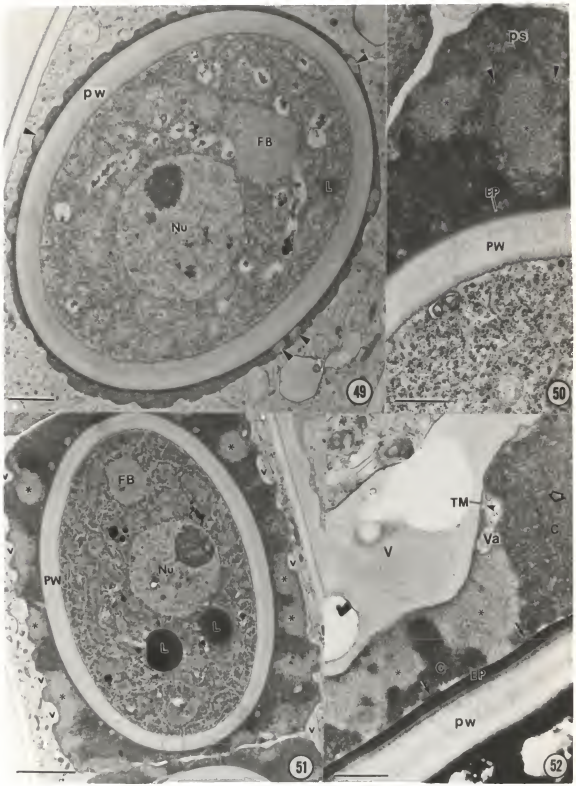
Figs. 2.49-2.52 Transmission electron micrographs of spore ontogeny in Scutellinia pennsylvanica.

Fig. 2.49 A young ascospore with less opaque zones (arrow heads) easily distinguishable in the perisporic sac (bar=1 μm). X 13,000.

Fig. 2.50 Gradual condensation in the perisporic sac with less opaque (asterisks) finely fiamentous areas and electron dense areas with coarse, dense fibrils (arrow heads) (bar=0.5 μm). X 32,000.

Fig. 2.51 An ascospore with nulceus, lipid droplets, and fibrillar bodies in the sporoplasm and epiplasmic vacuoles associated with less electron opaque areas in the perisporic sac (asterisks) (bar=2 μm). X 8,500.

Fig. 2.52 Differentiation of the condensed area with "tubular" elements (hollow arrow) and less opaque area (asterisks) in the perisporic sac shows the invagination of investing membrane (arrow head) into the perisporic sac forming a vacuole, and translucent bands at the base of the condensed area (solid arrows) (bar=0.5 μm). X 32,000.


pw
FB
L
Nu
49
DS
EP
PW
50
FB
Nu
PW
L
51
TM
V
Va
C
EP
pw
52

Figs. 2.53-2.54 Transmission electron micrographs of spore ontogeny in Scutellinia pennsylvanica.

Fig. 2.53 Detailed view of the redistribution of secondary wall material in the perisporic sac. Note the tubular features (arrow heads) in the condensed area and finely filamentous features in the less opaque zone (asterisk) (bar=0.5 μm). X 40,000.

Fig. 2.54 Detailed view of the condensed area showing its tubular elements (arrow heads) and the appearance of electron translucent bands (arrows) at the bases of ornaments (bar=0.5 μm). X 40,000.

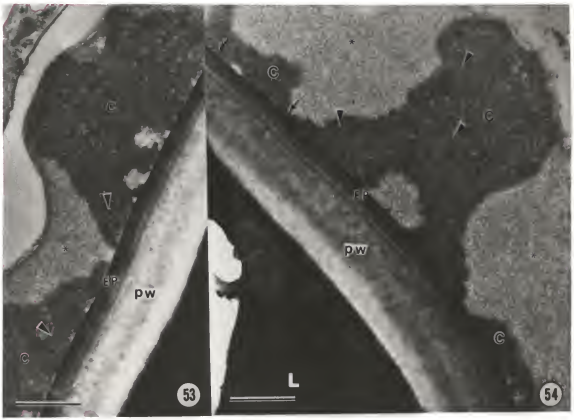

C
PP
pw
53
C
C
C
PP
pw
L
54

Fig. 2.55 Comparison of spore wall development in Cheilymenia, Coprobia and Scutellinia of the Ciliarieae.

a: outer investing membrane

b: primary wall

c: inner sporoplasma membrane

d: perisporic sac

e: condensed granule

f: less opaque, fibrillar area

g: epispore precursor

h: translucent line ready for the formation of second epispore layer

i: translucent zone

j: epispore layers

k: globular body

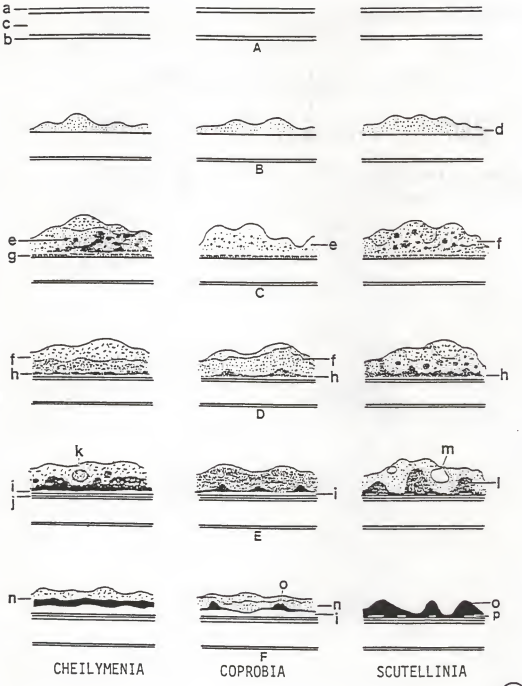
l: condensed area

m: vacuolation

n: spore sheath

o: ornament

p: translucent band.


a
c
b
A
d
B
e
g
e
f
C
f
h
f
h
h
D
k
i
j
m
i
l
E
n
o
n
i
o
p
F
CHEILYMENIA
COPROBIA
SCUTELLINIA

55

CHAPTER III
ASCOSPOROGENESIS IN SELECTED GENERA OF LACHNEAE

Introduction

The term "Lachnea" was used by Fries (1823) as a series name under the genus Peziza to include species producing hairy apothecia. The species under "Lachnea" included P.(=Mycolachnea) hemisphaerica Fr. and P.(=Sphaerosporella) brunnea Fr. Later, Boudier (1885) raised Fries' genus Peziza to the family level under which he accepted five tribes, including Lachneae (as "Lachnés"). Since Boudier (1885) used outer morphology to distinguish suborders, he separated cup fungi into Cupulés, Lenticlés and Mitrés. Similar groups of fungi producing hairy apothecia, therefore, were treated in different suborders just because of the different shapes of apothecia. For example, M. hemisphaerica (Wiggers ex Fr.) Fuckel, producing cupulate apothecia, was accepted in the tribe Lachnés of the family Pezizés (under Cupulés), while Trichophaea paludosa Boud. and Sphaerosporella brunnea (Alb. & Schw. ex Fr.) Svrcek & Kubicka, producing discoid apothecia were treated in the family Ciliariés (under Lenticulés). Subsequently, Boudier (1907) combined Ciliariés and Humariés families into Humariaceae and accepted them as two tribes, Ciliarieae and Humarieae.

Le Gal (1947) basically followed Boudier's system, but made a slight modification in the family Humariaceae to which she transferred the tribe Lachneae from the family Pezizés, combining it with the other two tribes previously recognized by Boudier (1907). Most of the fungi in her tribe Lachneae produce whitish or pale hairy apothecia and their paraphyses do not react blue with iodine. A similar treatment of Lachneae is basically followed by Rifai (1968) and Dennis (1978).

Arpin (1968) separated genera of Humariaceae based on the presence or absence of carotenoid pigments. Since carotenoid pigments were absent in taxa of Lachneae, he emended Otideaceae sensu Eckblad (1968) to accomodate the genera Sepultaria, Tricharia, Mycolachnea, Trichophaea, and Pseudobrophila. However, these genera were treated in the family Pyronemaceae by Eckblad (1968). Later, a new tribe Mycolachneae was proposed to replace Lachneae by Korf (1973), but it nevertheless accommodated basically the same group of fungi.

Rifai (1968) recognized four tribes of Humariaceae, the Aleurieae, Ciliarieae, Otideae and Lachneae. In our study, Rifai's system is temporarily followed and three genera of Lachneae are examined, i.e., Mycolachnea, Sphaerosporella and Trichophaea. The taxonomic position of Sphaerosporella is very controversial. It has been

recognized as a synonym of Humaria (=Mycolachnea) by Eckblad (1968) and was also merged with Trichophaea by other authors (Batra and Batra 1963). Because the name Humaria was used in many different senses and for different groups of fungi, Mycolachnea was proposed by Maire (1937) to replace it. We follow Maire's proposal in this paper.

A splendid study with the light microscope on the development of spore walls in various discomycetes was conducted by Le Gal (1947). She found in T. paludosa that the ornament consisted of callose and pectin, was of sporal origin, and arose directly from the primary wall.

The most extensive ultrastructural studies of ascosporogenesis in members of Lachneae were done by Merkus (1973, 1974, 1975, 1976). Mycolachnea and Trichophaea were studied by her and she concluded that the primary wall could be differentiated into epispore and endospore layers (Merkus 1974, 1976). However, sequences of spore wall ontogeny and ornament formation were not complete. In the two pyrophilic species, T. abundans (P. Karst.) Boud. and T. woolhopeia (Cooke & Phill.) Boud., striations in the outer part of the endospore were described. Merkus (1976) concluded that both genera shared the same pattern of ornament formation as Aleuria.

Previous research has shown that septal structures are highly correlated with spore ontogeny at generic and

familial levels (Wu and Kimbrough 1991a, 1991b). From our unpublished data, Sphaerosporella reveals different septal structures from those of Trichophaea and Mycolachnea, the latter genera possessing a septal plugging pattern similar to that of Aleuria (Kimbrough and Curry 1986b). The purpose of this paper is to describe the ultrastructure of spore ontogeny in these genera of Lachneae and attempt to correlate these data with other microscopic features. We also describe spore wall characters that appear thus far to be restricted to pyrophilic species only.

Material and Methods

Fresh collections were processed for transmission electron microscopic study following the procedures described by Curry and Kimbrough (1983). The following specimens were observed: Mycolachnea hemisphaerica (Wiggers ex. Fr.) Fuckel (FLAS F54599), on sandy soil among mosses at the base of live oak at The American Bank, NW 13th St. and 18th Ave., Gainesville, Florida, collected by J. Gibson on August 31, 1985; (unaccessioned), on soil, in University of Florida Horticultural Farm, Gainesville, Florida, collected by D. Samuelson, in July 1978; Sphaerosporella brunnea (Alb. & Schw. ex Fr.) Svrcek & Kubicka (FLAS F55461), on sandy soil, open field in forest near Sugarfoot Apt., Alachua Co., Gainesville, Florida, collected by C.-G.

Wu, on February 21, 1990; (unacessioned), on soil, E. side of Newnan's Lake, Alachua Co., Gainesville, Florida, collected by D. Samuelson, on February 26, 1982; Trichophaea paludosa Boud. (FLAS F55637), on soil bank by the walkway, in Devil's Millhopper St. Park, Alachua Co., Gainesville, Florida, collected by C.-G. Wu, on July 24, 1989.

Plastic blocks were sectioned on an LKB Huxley ultramicrotome with a diamond knife. After poststaining with uranyl acetate and lead citrate, the sections were examined at 60 kv, on a JEOL 100-CX electron microscope. Dry specimens were coated by gold and examined on Hitachi S-450 and S-4000 scanning electron microscopes.

Results

Mycolachnea hemisphaerica

After the delimitation of haploid nuclei forming young ascospores in the asci, an electron translucent primary wall is formed between the spore delimiting membranes (Figs. 3.1, 3.3). At this stage, some lipid drops are synthesized already in the sporoplasm (Fig. 3.1). Thereafter, electron dense secondary wall material is ususally found as clumps in the slightly dilated perisporic sac (Figs. 3.2, 3.4, 3.5). This dense material later spreads evenly over the primary wall and initiates the formation of the epispore layer (Fig. 3.6). Accompanying

this, crystal-like inclusion bodies are found in the sporoplasm (Figs. 3.4, 3.5). In the central area of these bodies, lattice structures are often present which are surrounded by reticulate filaments and enclosed by a membrane. Also within the sporoplasm, other membrane-bound bodies containing some electron opaque material are associated with the primary wall (Figs. 3.6, 3.7). After the appearance of secondary wall material in the perisporic sac, vacuolation in the epiplasm takes place (Fig. 3.2).

When the perisporic sac is fully expanded, secondary wall material is unevenly distributed (Figs. 3.8-3.10). Most of the wall material accumulates around the primary wall where the inner part is transformed into an epispore layer (Figs. 3.9, 3.10). The epispore is composed of a continuous row of radial, spinelike fibrils which appear to polymerize to form alternating periclinal layers (Fig. 3.9). The residues of unevenly distributed wall material in the perisporic sac soon forms electron dense granules which later coagulate into large clumps (Figs. 3.10-3.12). These clumps finally attach to the epispore forming ornaments (Fig. 3.13).

When more secondary wall material condenses within the periporic sac forming larger clumps on the epispore and the electron density of the primary wall begins to change. The outer part of primary wall becomes dense and zonate (Figs.

3.14, 3.16, 3.17) and shows spinelike striation perpendicular to the epispore (Figs. 3.16, 3.17). The epiplasm becomes completely vacuolate and the perisporic sac collapses onto the secondary wall (Figs. 3.14, 3.15). Numerous tuberculate protrusions are formed on the surface of ornaments (Figs. 3.15, 3.18, 3.19).

Excluding the crystal-like inclusion bodies and primary wall-associated bodies, a third type of dumbbell-shaped inclusion body with electron dense contents is found in the nucleoplasm and sporoplasm (Figs. 3.20, 3.21). Both crystal-like and dumbbell inclusion bodies appear to be formed first in the nucleoplasm and later pass through openings in the nuclear envelope into the sporoplasm (Figs. 3.22-3.25). When crystal-like bodies are released into the sporoplasm, they are often found associated with multivesicle bodies (Figs. 3.26, 3.27). They appear to be associated with rough ER (Figs. 3.25, 3.26, 3.27). The crystal-like body is actually composed of many interconnected tubular structures (Fig. 3.28, arrow heads). The lattice feature may disappear and become a dense clump (Figs. 3.27, 3.29, arrow head). Dumbbell bodies, after released into sporoplasm, may be associated with nuclear membranes or with the sporoplasma membrane (Figs. 3.21, 3.30). All of these inclusion bodies are only detected at

the early stages of spore ontogeny (Figs. 3.20, 3.30) and disappear after the ornaments form (Figs. 3.12, 3.13).

Sphaerosporella brunnea

The epiplasm of the young ascus contains numerous lipid droplets and some vacuoles within which dense bodies are included (Fig. 3.31). During meiosis, synaptonemal complexes are found in the nucleoplasm (Fig. 3.31, arrow heads). When meiosis is complete, spore delimiting membranes enclose each haploid nucleus and an electron translucent primary wall is formed between membranes (Figs. 3.32, 3.33). Lipid droplets and vacuoles are already detected in the sporoplasm of the ascospore (Fig. 3.32). Electron dense globules are usually found in the epiplasm near the primary wall and seem to be connected with endoplasmic reticulum (Fig. 3.33). A perisporic sac is formed immediately as the outer investing membrane expands (Fig. 3.34). Initially, the contents of early perisporic sac are sparse, loosely fibrillar material (Fig. 3.34), but later areas of dense clumps form as more and more material is deposited (Fig. 3.35). This dense material is finally distributed evenly on the primary wall forming epispore layers (Fig. 3.36).

The precursors of the epispore layer are radial spinelike fibrils. Many additional periclinal fragments are formed perpendicular to these spinelike precursors

(Fig. 3.36) and are later connected into smooth layers (Figs. 3.37, 3.38). After two layers of epispore are formed, a thin and barely detected band is synthesized above the epispore (Figs 3.37, 3.38, arrow heads), and a translucent zone is formed separating the perisporic sac from the epispore (Figs. 3.40, 3.41). At this stage, condensation of secondary wall material takes place and electron dense clumps are formed in the perisporic sac (Fig. 3.39).

After formation of a translucent zone in the perisporic sac, radial bands of electron dense material appear under the epispore layers and project into the primary wall (Fig. 3.42). These projecting bands exhibit a reticulate feature when viewed in grazing section (Fig. 3.45, asterisk). Periclinal lines are still detectable in these bands (Fig. 3.42, arrow heads). The area occupied by these intruding bands eventually becomes electron dense (Fig. 3.44). Once the outer zone of primary wall becomes electron dense, the radial bands protrude further into the wall (Fig. 3.44). The first formed layer of radial bands is afterward converted into a periclinally laminated layer (compare Fig. 3.44, S1 and Fig. 3.46, S1). Thus, in the mature stage, the spore wall is zonate and striated (Figs. 3.49, 3.50). Even though separation of the perisporic sac from epispore occurs, condensation of secondary wall

material continues in the perisporic sac (Figs. 3.43, 3.45, arrow heads). Large electron dense clumps are frequently found in the perisporic sac (Fig. 3.43). Within the homogeneous matrix of secondary wall material areas of uneven distribution of this material occurs, resulting in pale spots which are subsequently transformed into vacuoles (Figs. 3.44, 3.47). With vacuolation and disintegration of its contents, and thickening of the translucent zone, the perisporic sac becomes thinner (Fig. 3.49). Finally, the perisporic sac totally disappears, leaving ascospores surrounded by vacuoles (Fig. 3.50). The mature ascospore is very smooth and contains a large lipid guttule (Figs. 3.48, 3.50).

Trichophaea paludosa

Preceding wall formation of ascospores, the haploid nucleus is enclosed by two unit membranes, i.e. outer and inner spore delimiting membranes (Fig. 3.51). Subsequently, an electron translucent primary wall is deposited between the spore delimiting membranes (Figs. 3.52, 3.53). Lipid droplets are already present in the sporoplasm and inclusion bodies are found along the sporoplasma membrane associated with the primary wall (Fig. 3.55). The outer investing membrane is thereafter dilated, forming a perisporic sac within which secondary wall

material is deposited and soon used to form an epispore layer (Figs. 3.54, 3.56, 3.57). Large clumps of electron dense material accumulate on the primary wall (Fig. 3.56), but soon become evenly spread around the spores (Fig. 3.57). Once formation of the epispore layer is complete, more secondary wall material is deposited and accumulated near the epispore, resulting in two separate zones in the perisporic sac (Figs. 3.58, 3.59). Numerous dense bumps are formed from the sporoplasm and attached to the primary wall (Fig. 3.59). The inner part of the primary wall remains fibrillar (Fig. 3.61). Condensation of secondary wall material occurs in the inner zone of the perisporic sac within which some dense granules are formed (Fig. 3.61, asterisks). Functioning as nuclei, dense granules attract more material, forming larger clumps (Figs. 3.60, 3.62, 3.63). Near the final stage of wall formation, only large clumps of loosely arranged electron dense material accumulate to form ornaments on the epispore (Figs. 3.64-3.67). In the mature ascospore, there is a zonate primary wall surrounded by densely stained epispore layers which are covered by an ornamented secondary wall (Figs. 3.68, 3.69). With the aid of a scanning electron microscope, warted ornaments decorated with blunt spines are shown loosely distributed on the ascospores (Figs. 3.70-3.72).

Discussion

Mycolachnea and Trichophaea show basically an identical type of spore ontogeny (Fig. 3.73). Condensation of secondary wall material occurs only in the inner zone of the perisporic sac and is different from that found in Ascobolus (Wu and Kimbrough, 1991a, 1991b), Aleuria (Chapter I), Scutellinia and Cheilymenia (Chapter II) in which the condensation occurs evenly in every part of the perisporic sac. The type of wall or ornament formation of Mycolachnea and Trichophaea is thought to be a transitional stage between direct precipitation as found in Peziza (Dyby and Kimbrough, 1987), Helvella (Gibson and Kimbrough, 1988), and Morchella (unpublished data) and typical gradual condensation as found in Ascobolus (Wu and Kimbrough, 1991a, 1991b), Aleuria, Scutellinia, and Cheilymenia (Chapter I and II). Because condensation only occurs in the area closer to the epispore layer, if secondary wall material coagulates tightly and much closer to the epispore layer, it could directly form ornamentation on the epispore surface and become the type of direct precipitation as found in Helvella and Peziza. Besides similarity in spore ontogeny, both Mycolachnea and Trichophaea produce sporoplasma membrane-associated dense bodies which could contribute to primary wall formation (Fig. 3.61). The increase of primary wall thickness is only found thus far

in Tarzetta (Wu, 1990) and Ascobolus (Wu and Kimbrough 1991a, 1991b).

Although Mycolachnea and Trichophaea have the same type of spore ontogeny, other characters may be used to distinguish these two genera. For instance, Mycolachnea produces cupulate, but Trichophaea forms discoid apothecia. Additionally, Mycolachnea produces more varied types of inclusion bodies such as dumbbell bodies and crystal-like bodies in the nucleoplasm. Dumbbell bodies may be released into the sporoplasm and contact with the primary wall, and are possibly the precursors of sporoplasma membrane-associated dense bodies. Since a nucleolus extruded into the cytoplasm is one of the common phenomena in the cell division, dumbbell body is possibly the residue of extruded nucleolus (Heath, 1978). Even though the function of crystal-like bodies is still unknown, their disintegration into dense material in the sporoplasm is observed (Figs. 3.27, 3.29). The relationship among all of these inclusion bodies is unclear. However, they are only found in early spore development before the ornaments are totally formed. These observations seem to support the hypothesis that they are involved in the spore wall formation such as was found in the genera Ascobolus (Wu and Kimbrough, 1991a, 1991b), Cheilymenia, and Scutellinia (Chapter II).

In this study, we agree partly with Le Gal's (1947) observation on T. paludosa that some aspect of wall formation is contributed by the sporoplasm. We could not confirm, however, that the secondary wall (ornament) arises directly from the primary wall and is of spore origin. Nevertheless, data from these and other studies show that compounds forming the primary wall are derived from the sporoplasm (Fig. 3.61). Most species of Trichophaea produce smooth ascospores, but T. paludosa is one of the species forming warted spores whose ornaments, however, are easily dissolved in 2.5% KOH (Kanouse, 1958). The loose matrix of secondary wall material seen at the ultrastructural level (Figs. 3.66, 3.67) may account for the fragile nature of spore ornaments in this species.

Although S. brunnea produces smooth ascospores, it still shares the same type of spore ontogeny found in Trichophaea and Mycolachnea (Fig. 3.73). In Sphaerosporella, however, epispore layers and the perisporic sac are later separated by a translucent zone. Even though the condensation of secondary wall material still occurs in the detached perispore, it does not attach to the epispore to form a thickened secondary wall or ornaments. The formation of a detached perispore may explain the presence of a loose coating which causes ascospores to appear asperate in certain taxa (Rifai,

1968). In addition to the differentiation of a translucent zone, the radial electron dense bands intruding into the primary wall obviously result from chemical changes in the outer primary wall. It would appear from staining reactions that compounds from the perisporic sac continue to diffuse through the epispore layer and cause further chemical changes in the outer layer of primary wall. This radiate banding of the primary wall seems to be associated with pyrophilic fungi only and may have evolved for high temperature resistance. A similar banding has been found in the T. woolhopeia, T. abundans, Anthracobia melaloma (Alb. & Schw. ex Pers.) Boud. (Merkus, 1974), and Octospora (Chapter I). These species have been frequently recorded in burnt areas or on recent volcanic deposits (Petersen, 1970, 1971, 1975; Carpenter et al. 1982). In this study, however, T. paludosa and M. hemisphaerica were also shown to have this kind of structure in the primary wall, although these species are not always associated with a pyrophilic habitat.

Some similarities have been found between pyrophilic and coprophilous fungi such as gradual condensation of secondary wall material and the presence of a translucent zone. Results from our EM studies of septal structures (Kimbrough and Curry, 1986b) also suggest that a strong connection exists between pyrophilic and coprophilous

Pezizales. The taxonomic significance of these data will be treated in a later publication.

CHAPTER III

Abbreviations used in the figures:

AW:	ascal wall
C:	clumps of secondary wall material
DB:	dense body
DG:	dense globule
DLB:	dumbbell body
EP:	epispore
EP1:	first layer of epispore
EP2:	second layer of epispore
ER:	endoplasmic reticulum
F:	fibrillar wall
IB:	crystal-like inclusion body
IM:	investing membrane
L:	lipid droplet
M:	mitochondria
MVB:	multivesicular body
NP:	nuclear pore
Nu:	nucleus
O:	spore ornament
PS:	perisporic sac
PW:	primary wall
S:	spinelike intruding structures
S1:	first layer of spinelike structure
S2:	second layer of spinelike structure
SDM:	spore delimiting membrane
SM:	sporoplasma membrane
SPM:	sporoplasm
SWM:	secondary wall material
TM:	tonoplast membrane
TZ:	translucent zone
V:	vacuole.

Figs. 3.1-3.7 Transmission electron micrographs of spore ontogeny in Mycolachnea hemisphaerica.

Fig. 3.1 A young ascospore with translucent primary wall and showing a lipid droplet and nucleus in the sporoplasm (bar=2 μm). X 8,000.

Fig. 3.2 Secondary wall material coagulates in clumps (arrow head) on the primary wall. Note the appearance of vacuoles in the epiplasm (bar=2 μm). X 8,000.

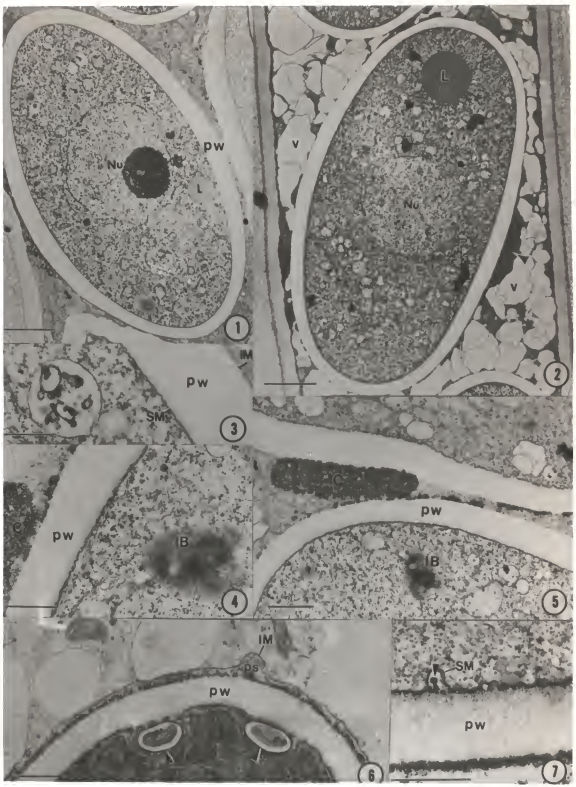
Fig. 3.3 Detail view of primary wall delimited by investing and sporoplasma membranes (bar=0.5 μm). X 32,000.

Fig. 3.4 Detailed view of secondary wall material deposition and crystal-like inclusion body (bar=0.5 μm). X 33,000.

Fig. 3.5 Secondary wall material deposited on primary wall and the presence of crystal-like inclusion body in the sporoplasm (bar=1 μm). X 14,000.

Fig. 3.6 Sporoplasma membrane-associated inclusion bodies (arrow heads) (bar=0.5 μm). X 30,000.

Fig. 3.7 Small vesicles with dense contents are released from sporoplasma membrane into primary wall (bar=0.5 μm). X 50,000.


pw
Nu
L
1
v
L
Nu
v
2
IM
pw
SM
3
pw
IB
4
pw
IB
5
IM
Ds
pw
6
SM
pw
7

Figs. 3.8-3.13 Transmission electron micrographs of spore ontogeny in Mycolachnea hemisphaerica.

Fig. 3.8 Secondary wall material is deposited in a slightly dilated peripsoric sac (bar=1 μm). X 11,000.

Fig. 3.9 Secondary wall material coagulate around the area closer to the epispore (bar=0.5 μm). X 30,000.

Fig. 3.10 Sporoplasma membrane-associated dense bodies (arrow heads) and gradual condensation of wall material (asterisks) in the perisporic sac (bar=0.5 μm). X 34,000.

Fig. 3.11 Clumps of condensed wall material (arrow heads) randomly distributed in the perisporic sac (bar=2 μm). X 7,500.

Fig. 3.12 More condensed clumps congeal into larger globules (arrow heads) (bar=1 μm). X 12,500.

Fig. 3.13 Condensed clumps attach to epispore in the formation of ornaments (bar=0.5 μm). X 25,000.

L
pw
ps
8
PS
ER
pw
9
EP
ps
pw
V
10
L
Nu
11
L
12
PS
13

Figs. 3.14-3.17 Transmission electron micrographs of spore ontogeny in Mycolachnea hemisphaerica.

Fig. 3.14 Secondary wall material condenses into clumps in the perisporic sac. Note the electron density change in the outer primary wall and complete vacuolation of the epiplasm (bar=0.5 μm). X 40,000.

Fig. 3.15 Tuberculate spore ornaments (arrow heads) (bar=0.5 μm). X 40,000.

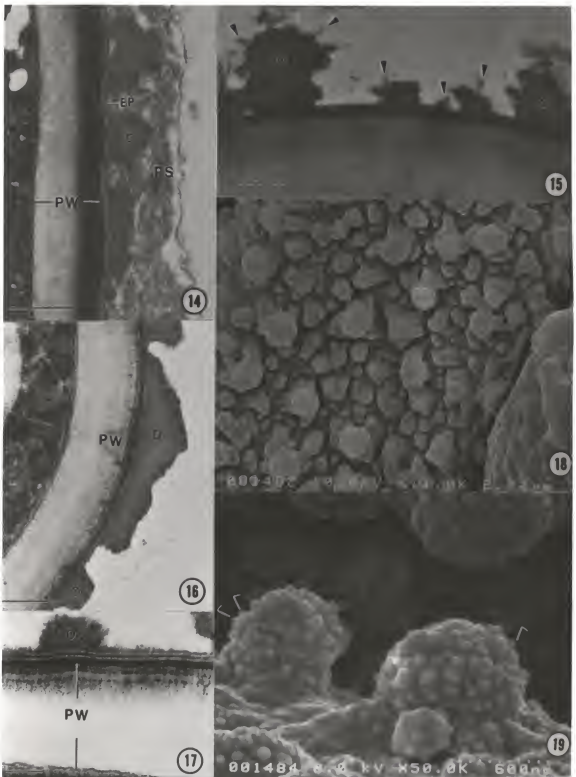
Fig. 3.16 Wall structures in the mature ascospore. Note the radial and periclinal banding in the primary wall (bar=0.5 μm). X 38,000.

Fig. 3.17 Detailed view on primary wall showing zonation and spiny feature (bar=0.2 μm). X 60,000.

Figs. 3.18-3.19 Scanning electron micrographs of ascospores of Mycolachnea hemisphaerica.

Fig. 3.18 Ornamented spore surface (bar=2.72 μm). X 11,000.

Fig. 3.19 Detailed view of the tuberculate surface of ornaments (bar=600 nm). X 50,000.


EP
PS
PW
14
PW
16
PW
17
15
18
19
001484 8.0 kV X50.0K 600nm

Figs. 3.20-3.25 Transmission electron micrographs of sporoplasmic inclusions of Mycolachnea hemisphaerica.

Fig. 3.20 Dumbbell and crystal-like inclusion bodies are present in the nucleoplasm and sporoplasm of a very young spore (bar=2 μm). X 9,000.

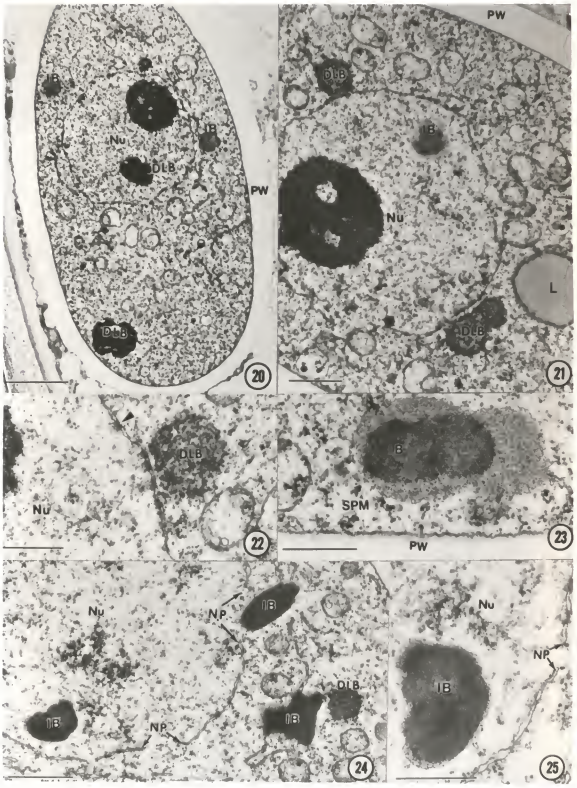
Fig. 3.21 Dumbbell bodies released into the sporoplasm but attached to the nuclear membranes. Note the presence of crystal-like inclusion body (IB) in the nucleoplasm (bar=1 μm). X 16,000.

Fig. 3.22 Dense dumbbell body is found next to the nuclear pores (arrow head) (bar=0.5 μm). X 38,000.

Fig. 3.23 Detailed view of sporoplasmic crystal-like inclusion body which is surrounded by reticulate membraneous structures (bar=0.5 μm). X 45,000.

Fig. 3.24 Crystal-like bodies released from a nucleus. Note the dialation of nuclear pores (arrows) (bar=1 μm). X 20,000.

Fig. 3.25 Detailed view of nuclear crystal-like body. Note the opening of nuclear pore (arrows) (bar=0.5 μm). X 50,000.


IB
Nu
DLB
PW
20
PW
DLB
IB
Nu
L
DLB
21
DLB
Nu
22
IB
SPM
PW
23
Nu
NP
IB
DLB
IB
IB
NP
24
Nu
NP
IB
25

Figs. 3.26-3.30 Transmission electron micrographs of sporoplasmic inclusions of Mycolachnea hemisphaerica.

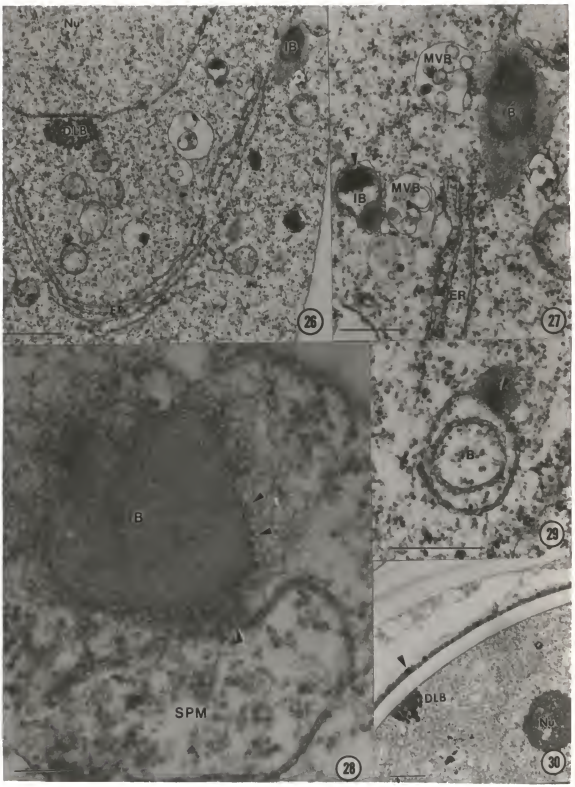
Fig. 3.26 Dumbbell body and crystal-like body are present in the sporoplasm (bar=1 μm). X 20,000.

Fig. 3.27 Detailed view of crystal-like bodies associated with multivesicular bodies in the sporoplasm. Note the degeneration of crystal-like body (arrow head) into a dense material which is still surrounded by reticulate membraneous structures (bar=0.5 μm). X 42,000.

Fig. 3.28 Detailed view of crystal-like body showing interconnceted tubulular structures (arrow heads) (bar=0.2 μm). X 90,000.

Fig. 3.29 Degenerating crystal-like body in the sporoplasm. Note the presence of circular structure and dense material within the body (bar=0.5 μm). X 60,000.

Fig. 3.30 Dumbbell body is associated with primary wall. Note that condensation of wall material occurs in the perisporic sac (bar=1 μm). X 11,000.


Nu
IB
DLB
ER
26
MVB
B
IB
MVB
ER
27
IB
SPM
28
B
29
DLB
NC
30

Figs. 3.31-3.35 Transmission electron micrographs of spore ontogeny in Sphaerosporella brunnea.

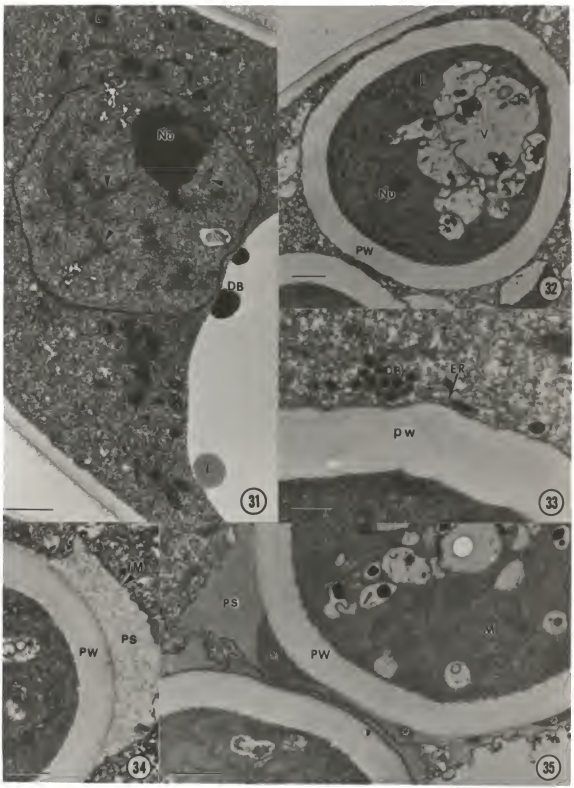
Fig. 3.31 A meiosing diploid nucleus with synaptonemal complexes (arrow heads). Note the presence of vacuoles with dense bodies inside the epiplasm (bar=1 μm). X 15,000.

Fig. 3.32 A young ascospore encircled only by primary wall (bar=1 μm). X 10,000.

Fig. 3.33 Detailed view of primary wall and some ER-associated dense bodies (bar=0.5 μm). X 26,000.

Fig. 3.34 The outer investing membrane dilates to form a perisporic sac. Note early deposition of epispore precursors (bar=1 μm). X 13,000.

Fig. 3.35 More secondary wall material deposited in the perisporic sac with dense clumps (asterisks) appearing on the primary wall (bar=1 μm). X 16,000.


Nu
DB
31
Nu
V
PW
32
DB
ER
pw
33
M
PS
PW
34
PS
PW
M
35

Figs. 3.36-3.42 Transmission electron micrographs of spore ontogeny in Sphaerosporella brunnea.

Fig. 3.36 The early stage of epispore layer formation showing both radial and periclinal striations (bar=0.5 μm). X 32,000.

Fig. 3.37 Two layers of epispore showing its spinelike precursors and some secondary wall material (arrow head) condensed above the epispore (bar=0.5 μm). X 52,000.

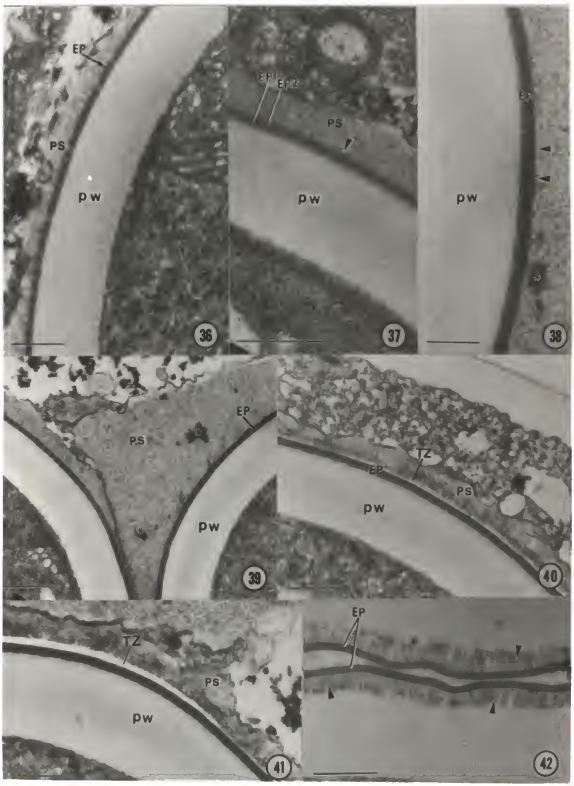
Fig. 3.38 A narrow dense wall band formed on epispore layers while condensed wall clumps (asterisk) develop in the perisporic sac (bar=0.25 μm). X 66,000.

Fig. 3.39 Radom condensation of secondary wall material (asterisks) occurs in the perisporic sac (bar=0.5 μm). X 26,000.

Fig. 3.40 The appearance of a translucent zone between the epispore and perisporic sac (bar=0.5 μm). X 32,000.

Fig. 3.41 Separation of the perisporic sac from the epispore by thickening translucent zone (bar=0.5 μm). X 40,000.

Fig. 3.42 Radial dense bands with periclinal striation (arrow heads) intruding into the primary wall from the epispore (bar=0.5 μm). X 40,000.


EP
PS
pw
36
EP1
EP2
PS
pw
37
EP
pw
38
EP
PS
pw
39
TZ
EP
PS
pw
40
TZ
PS
pw
41
EP
42

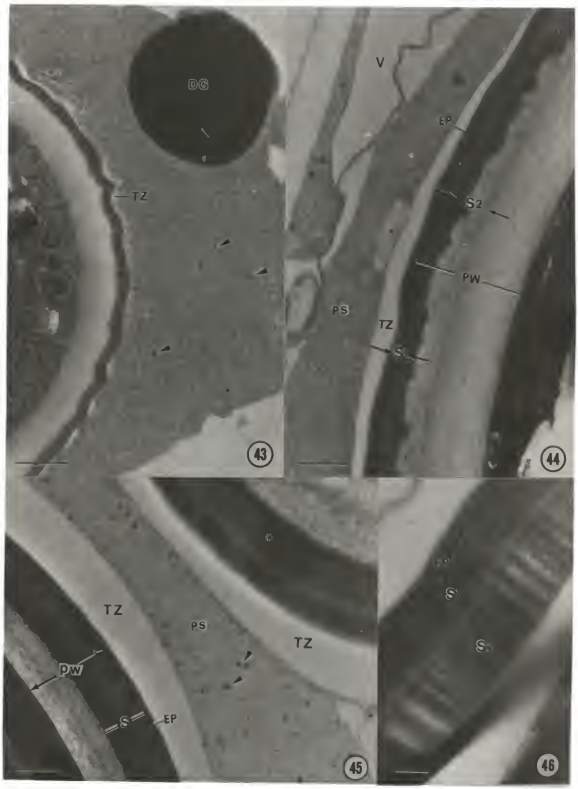
Figs. 3.43-3.46 Transmission electron micrographs of spore ontogeny in Sphaerosporealla brunnea.

Fig. 3.43 Random condensation of wall material (arrow heads) continues even after the translucent zone appears. Note the large dense globule is found frequently in the perisporic sac (bar=1 μm). X 16,000.

Fig. 3.44 Two layers of radial banding are differentiated in the primary wall and the electron density also changes from S1 to S2. Note the formation of electron translucent spots (asterisk) in the perisporic sac (bar=0.5 μm). X 32,000.

Fig. 3.45 Reticulate feature (asterisk) of primary wall banding as seen in grazing section. Note the radom condensation in the perisporic sac (arrow heads) (bar=0.5 μm). X 26,000.

Fig. 3.46 Detailed view of two layers of banded primary wall. Note S1 layer has become laminated (bar=0.1 μm). X 100,000.


DG
TZ
43
V
EP
S2
PW
PS
TZ
S
44
TZ
PS
TZ
PW
S
EP
45
S
S
46

Figs. 3.47-3.50 Transmission electron micrographs of spore ontogeny in Sphaerosporella brunnea.

Fig. 3.47 Vacuolated perisporic sac (arrow heads) is highly reduced and dense bodies (asterisks) are attached to the perisporic sac (bar=1 μm). X 13,000.

Fig. 3.48 A globular ascospore with a large lipid drop is surrounded by giant vacuoles (bar=2 μm). X 10,000.

Fig. 3.49 A mature ascospore covered by a highly reduced perisporic sac which is separated from the epispore by a wide translucent zone (bar=0.5 μm). X 40,000.

Fig. 3.50 Wall of mature ascospore fully exposed to vacuole after the perisporic sac disappears (bar=0.5 μm). X 52,000.

TZ
47
V
V
48
PS
TZ
49
AW
50

Figs. 3.51-3.56 Transmission electron micrographs of spore ontogeny in Trichophaea paludosa.

Fig. 3.51 A young ascospore surrounded only by spore delimiting membranes (bar=1 μm). X 14,000.

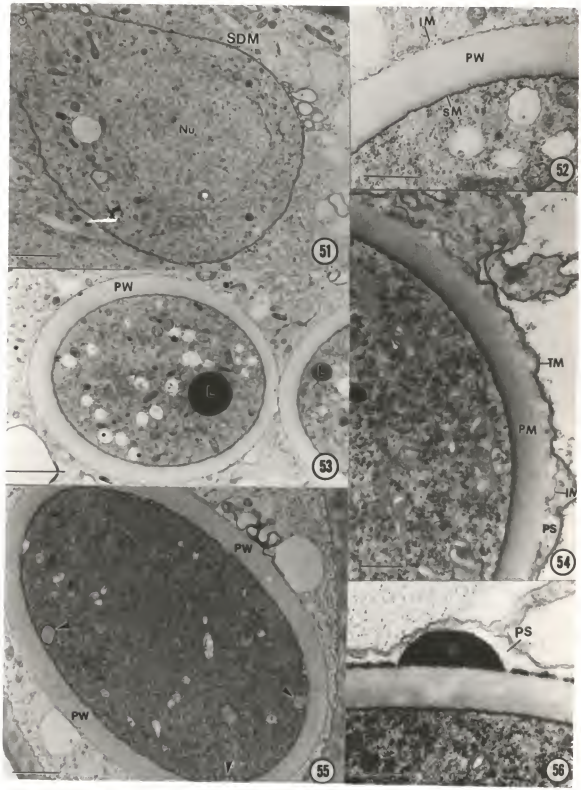
Fig. 3.52 A translucent primary wall delimited by an outer investing and an inner sporoplasma membranes (bar=0.5 μm). X 34,000.

Fig. 3.53 A large lipid drop is shown in the sporoplasm of a young ascospore (bar=2 μm). X 10,000.

Fig. 3.54 The outer investing membrane dilates to form a perisporic sac in which secondary wall material is deposited (bar=0.5 μm). X 26,000.

Fig. 3.55 Membrane-bound bodies (arrow heads) are included by the sporoplasma membrane (bar=2 μm). X 7,000.

Fig. 3.56 Clumps of secondary wall material condense before epispore formation (bar=0.5 μm). X 30,000.


SDM
Nu
51
IM
PW
SM
52
PW
53
TM
PM
IN
PS
54
PW
PW
55
PS
56

Figs. 3.57-3.61 Transmission electron micrographs of spore ontogeny in Trichophaea paludosa.

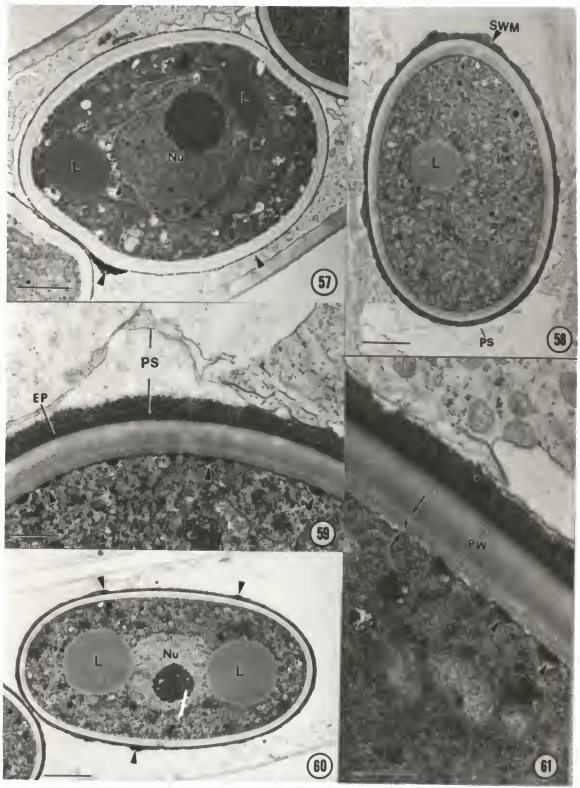
Fig. 3.57 Secondary wall material (arrow heads) condenses around primary wall to form the epispore (bar=2 μm). X 8,000.

Fig. 3.58 Condensed wall material accumulates but is still confined near primary wall (bar=2 μm). X 7,000.

Fig. 3.59 Detailed view of the distribution of secondary wall material in the perisporic sac. Note the presence of sporoplasma membrane-associated dense bodies (arrow heads) (bar=0.5 μm). X 30,000.

Fig. 3.60 A maturing spore with unevenly distributed secondary wall material (arrow heads) and two large lipid drops within (bar=2 μm). X 7000.

Fig. 3.61 Inner part of primary wall associated with dense bodies is fibrillar (arrow heads) while dense granules (asterisks) appear in the perisporic sac (bar=0.5 μm) . X 40,000.


L
L
Nu
57
SWM
L
PS
58
PS
EP
59
PW
61
L
Nu
L
60

Figs. 3.62-3.67 Transmission electron micrographs of spore ontogeny in Trichophaea paludosa.

Fig. 3.62 Radom condensation of secondary wall material (arrow heads) in the perisporic sac (bar=2 μm). X 7,500.

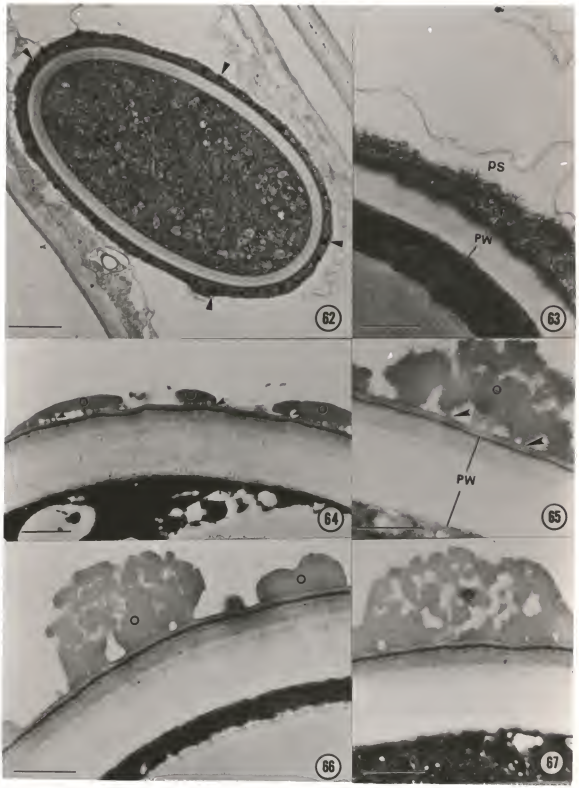
Fig. 3.63 Two layers of epispore are formed and the outer part of primary wall becomes electron opaque (bar=0.5 μm). X 38,000.

Fig. 3.64 More electron dense material condenses into large ornaments but appear loosely attached (arrow heads) to the epispore layer (bar=0.5 μm). X 30,000.

Fig. 3.65 Detailed view of the loose attachment (arrow heads) of secondary wall (bar=0.5 μm). X 40,000.

Fig. 3.66 Further condensation of secondary wall material into ornaments (bar=0.5μm). X 38,000.

Fig. 3.67 Unequal distribution of wall material before the ornaments are formed (bar=0.5 μm). X 38,000.


62
PS
PW
63
64
PW
65
66
67

Figs. 3.68-3.69 Transmission of electron micrographs of spore ontogeny in Trichophaea paludosa.

Fig. 3.68 Mature ascospore with two large lipid drops and dense sporal contents (bar=5 μm). X 6,000.

Fig. 3.69 Mature spore wall and ornaments (bar=0.2 μm). X 62,000.

Fig. 3.70-3.72 Scanning electron microgarphs of ascospores in Trichophaea paludosa.

Fig. 3.70 Ascospore is enclosed by an investing membrane covering ornamented surface (bar=3 μm). X 10,000.

Fig. 3.71 Loosely ornamented ascospores (bar=5 μm).

Fig. 3.72 Detailed view of ornament surface showing rounded protrusions (bar=1.2 μm).

L
L
68

M
70

PW
69

71
72

Fig. 3.73 Comparision of spore ontogeny in Mycolachnea, Sphaerosporella and Trichophaea.

a: investing membrane.

b: sporoplasma membrane.

c: perisporic sac.

d: primary wall.

e: dense secondary wall material.

f: epispore precursors.

g: sporoplasma membrane-associated dense body.

h: dense granules.

i: epispore layer.

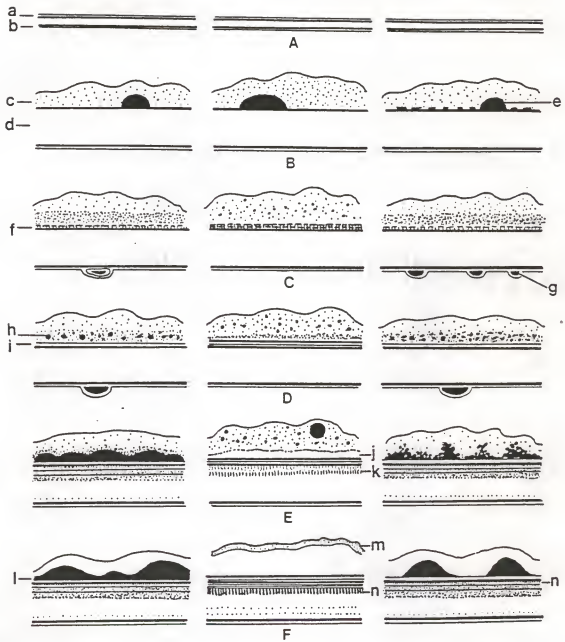
j: translucent zone.

k: precursors of dense intruding bands.

l: mature ornament.

m: reduced perisporic sac.

n: dense intruding bands.


a
b
A
c
d
e
B
f
C
g
h
i
D
j
k
E
l
m
n
n
F
Mycolachnea
Sphaerosporella
Trichophaea

73

CHAPTER IV
ASCOSPOROGENESIS IN SELECTED GENERA OF OTIDEAE-
CYTOLOGICAL COMPARISON BETWEEN Otidea and Tarzetta

Introduction

The tribe Otideae (Humariaceae) was generally characterized as producing cupulate apothecia, but without stiff hairs and carotenoids (Le Gal, 1947; Dennis, 1978). Based on different concepts in the evaluation of characters, genera of Otideae were treated in different families such as Aleuriaceae (=Pezizaceae) (Le Gal, 1947), Humariaceae (Rifai, 1968), Pyronemataceae (Korf, 1973), and Pezizaceae (Seaver, 1942; Dennis, 1978). But Eckblad (1968) raised the tribe to family status, Otideaceae, emphasizing the non-carotenoid and glabrous apothecia as major characters for the family and placed there Geopyxis, Otidea, Pustulina (=Tarzetta), Sowerbyella, and Ascosparassis. In the same year, Arpin (1968) emended Eckblad's Otideaceae as a group of non-carotenoid genera and included the genera Sepultaria, Tricharia, Mycolachnea, Pseudombrophila and Trichophaea. Arpin's Otideaceae was not accepted widely (Kimbrough, 1970; Dennis, 1978; Korf, 1973).

In the Otideae, Geopyxis, Otidea and Tarzetta are commonly collected in North America. The ultrastructure of

Geopyxis was studied thoroughly (Kimbrough and Gibson, 1989) and its septal structures and spore ontogeny turned out to be similar to those of species of Helvella, even though Geopyxis does not produce tetranucleate ascospores or have apothecial features of Helvella. Additionally, Geopyxis produces eguttulate ascospores and brightly pigmented apothecia which make it easily distinguished from Otidea and Tarzetta.

The generic limit between Otidea and Tarzetta has been discussed by Harmaja (1974a). Otidea is generally defined as producing ear-shaped apothecia, or at least splitting down one side, and whose paraphyses are usually highly curved at the tip. However, there are a number of exceptions, since some speices of Otidea, such as O. indivisa and O. apophysata, may produce stipitate apothecia or sessile, cupulate as Tarzetta. The apices of paraphyses are not necessarily curved in all species of Otidea. Some species such as O. kauffmanii, O. rainierensis, and O. phlebophora form only slightly curved paraphyses (Harmaja, 1974b), but true curved paraphyses are found in species of Tarzetta such as T. spurcata and T. pusilla (Harmaja, 1974c). The most prominent difference between Otidea and Tarzetta is probably (i) the carminophylic nuclei found only in Tarzetta and (ii) the uninucleate cells of

paraphyses in Tarzetta which are always binucleate in Otidea.

Even though Merkus (1975) and Schrantz (1966) did spore ontogeny studies on these two genera, the descriptions and explanations on spore development were incomplete and some of the information was contrary to data derived from my investigations. For instance, both authors thought the epispore was differentiated from the primary wall, but I demonstrated in these studies that the epispore was formed directly from the secondary wall material in the perisporic sac (Wu and Kimbrough, 1991a, 1991b). This observation has been consistently found in the studies of other tribes of Humariaceae (Wu and Kimbrough, 1991a, 1991b, Chapter I, II, and III). Besides, through scanning electron microscopic studies on the ascospores of Otidea, I found the ascospores were slightly ornamented (Wu and Kimbrough, 1990) and not smooth as Merkus (1975) and other mycologists described (Seaver, 1942; Kanouse, 1949; Eckblad, 1968).

In this study, cytological and ultrastructural comparisons between Otidea and Tarzetta, including spore ontogeny and nuclear condition in the paraphyses, are conducted in order to clarify taxonomic confusion. These data will also be compared with those of other Humariaceae to determine the position of the Otideae within the family.

Material and Methods

Specimens were all collected from the field and after fixation, materials were embedded in Spurr's plastics following the procedure described by Curry and Kimbrough (1983). The following collections were observed: Otidea alutacea (Per.) Massee var. microspora Kanouse, on moist leaves and debris along stream near Rustic Falls Rd., 1/4 mi. beyond Rustic Falls, off Bull Pen Rd., 6 mi. S.E. of Highlands, Macon County., North Carolina, (FLAS 55426); on soil near Priest Lake, Bonner Co., Idaho, (unaccessioned); O. grandis (Pers.) Massee, on wood in fallen pine needles, ca. 4 miles SW of Highlands Biological Station, lower Blue Valley, Macon Co., North Carolina, (FLAS-F51073); O. leporina (Batsche ex Fr.) Fckl., under spruce, Big Lagoon Park, California, (FLAS-F22031); another sample, collection site unknown, California, (FLAS-F22031); O. onotica (Fr.) Fukl., on soil, by US 19, 3-4 mi. S. of Sutton, West Virginia, (FLAS 52959); on soil in mixed hardwoods & conifers in Van Neil Glades National Campground approximate 6 mi. W. of Highlands, Macon County, North Carolina, (FLAS 52921); Tarzetta cupularis (L. ex Fr.) Lambotte, on wash soil, subplot # 51, Pack Forest under Douglass fir, Seattle, Washington, (FLAS 55431); T. gaillardiana (Boud.) Korf & Rogers, on moist mossy soil of trail alongside Hwy

84, 8 mi., W. of Highlands, Macon County, North Carolina, (FLAS 55420).

Plastic blocks were sectioned on an LKB Huxley ultramicrotome with a diamond knife. After poststaining with uranyl acetate and lead citrate, the sections were examined at 60 kv, on a JEOL 100-CX electron microscope. Dry herbarium specimens were coated by gold and examined on a Hitachi S-4000 scanning electron microscope.

Results

Tarzetta cupularis

Spore delimiting membranes (SDM) are differentiated from the ascal plasma membranes in the ascus (Figs. 4.1-4.2). Accompanying SDM formation, haploid nuclei with condensed chromatin result from meiotic divisions. Thereafter, SDMs invaginate to enclose haploid nuclei and other organelles (Fig. 4.3). An electron-dense, thin initial primary wall is deposited between two SDMs (Figs. 4.4-4.5). At this stage, a number of electron dense globular bodies are present in the epiplasm (Figs. 4.4-4.5).

Immediately after the formation of initial wall, the outer investing membrane inflates (Fig. 4.5) and becomes a perisporic sac (Fig. 4.4). Inside the sporoplasm, a few smaller lipid droplets are produced which later coagulate into larger guttules at polar ends of spores. Numerous

tubular depressions are formed along the investing membrane (Figs. 4.6-4.7). Some dense globular material appears in the matrix of the perisporic sac and later spreads extensively on the surface of the initial wall (Fig. 4.8). This dense wall material is still interconnected by narrow dense lines (Fig. 4.9) and subsequently becomes a thin layer of the epispore. Dense bodies are also shown in the sporoplasm at this stage of wall differentiation (Figs. 4.8-4.9). Afterwards, a thicker, electron translucent wall is formed under the initial opaque wall (Figs. 4.10-4.11). At the same stage, additional dense material is present which again distributes evenly on the surface of the epispore layer (Figs. 4.10-4.13). More deposition of secondary wall material results in the formation of minutely ornamented spores (Figs. 4.14-4.15).

After formation of the translucent primary wall layer, two large lipid guttules are produced inside the sporoplasm (Fig. 4.14). The condensed chromatin is visible through all of the stages of spore ontogeny (compare Figs. 4.3 and 4.14).

Paraphyses of T. cupularis are uninucleate and they contains a slender crystal body which is closely associated with the nucleus (Figs. 4.28, 4.29). Crystal bodies are membrane-bound and show crystal-like lattice structure.

Tarzetta gaillardiana

During meiosis, spore delimiting membranes (SDMs) are not differentiated until eight haploid nuclei are produced. Condensed chromatin in the nuclei is clearly shown at this stage. Later on, SDMs soon enclose each nucleus (Figs. 4.16-4.17). In the primordial membrane-bounded ascospores, abundant inflated vesicles and endoplasmic reticulum are present (Fig. 4.17) and an electron-dense initial primary wall is formed between the SDMs (Fig. 4.18).

The outer investing membrane soon expands, forming a perisporic sac which contains loose fibrillar material (Fig. 4.18). At this stage, small lipid droplets and vacuoles are present throughout the sporoplasm (Figs. 4.18-4.19). As found in the previous species, a few tubular depressions are found near the outer investing membrane (Fig. 4.19). Electron dense globular bodies then appear in the perisporic sac (Fig. 4.19) and later spread along the primary wall forming epispore precursors (Fig. 4.20). The epispore precursors are usually arranged as periclinal fragments (Fig. 4.21) which are afterwards interconnected into a smooth epispore layer. About the time that a translucent wall layer is formed under the electron dense initial wall, some dense bodies appear in the sporoplasm (Fig. 4.22). Numerous dense globules from the epiplasm are

associated with the outer investing membrane (Fig. 4.22) and they appear to infiltrate into the perisporic sac.

After formation of the epispore layer, more secondary wall material is deposited sometimes as clumps attached to the epispore (Fig. 4.24). However, in mature ascospores, there is only limited secondary wall deposited onto the epispore (Fig. 4.23). There are always two polar guttules in the mature ascospores and the outer wall is uniformly thin throughout (Fig. 4.25).

Nuclear number in paraphyses and excipular tissue cells of T. gaillardiana is consistently singular and with condensed chromatin (Figs. 4.26-4.28). The nucleus is large enough to be easily seen with the light microscope.

Otidea alutacea var. microspora

O. grandis

O. leporina

O. onotica

Spore wall ontogeny in species of Otidea (Fig. 4.36 B & C) is very similar to that found in members of Aleurieae and Lachneae in that electron translucent primary wall material is deposited between the SDMs. Thereafter, the outer investing membrane dilates, forming the perisporic sac in which secondary wall material is deposited. Spine-like epispore precursors are then differentiated from this

wall material and later on interconnected into a smooth epispore layer. In the species O. alutacea var. microspora, O. grandis and O. onotica, secondary wall material inside the perisporic sac becomes more dense around the epispore than the outer periphery. The material of the inner zone gradually condenses into electron dense granules which later attach to the epispore layer forming the delicate but fragile reticulate ridges which are visible with SEM but only barely seen from light microscope (Figs. 4.31-4.34). In the transmission electron microscope, the reticulate ridges are composed of fibrillar material and not firmly consolidated (Fig. 4.33). The reticulate ridges on the spore surface appear to be more concentrated at the polar ends (Figs. 4.31, 4.32, 4.34, arrow heads). Different species are only distinguished by their spore size and outer shape.

Otidea leporina is different from the other three species of Otidea studied by the production of smooth ascospores (Fig. 4.35). After the formation of the epispore layer, an electron translucent zone is differentiated, separating epispore from the perisporic sac which finally degenerates and becomes a spore sheath (Figs. 4.35; 4.36 B).

Paraphyses of O. alutacea var. microspora and O. onotica are always binucleate, but do not contain condensed chromatin (Fig. 4.30).

Discussion

In this study, spore ontogeny at the generic level is confirmed again to be very consistent. Four species of Otidea were investigated by either transmission or scanning electron microscopy, and they all show the same type of spore ontogeny. Excluding O. leporina, ascospores of the other three species all bear reticulate ridges or folds which are composed of fragile wall material. This kind of verrucose wall surface has not been reported before (Kanouse, 1949; Eckblad, 1968). However, O. grandis was recorded to produce slightly roughed ascospores by Seaver (1942) and Kanouse (1949). This description agrees with my scanning electron microscopic data and also with my personal light microscopical observation. Although the ascospores of O. leporina are smooth and covered by a spore sheath (or aborted perisporic sac) as found in Sphaerosporella (Chapter III), spore ontogeny is exactly the same as the other species of Otidea and is referred to as the gradual condensation type (Fig. 4. 36 B).

O. onotica and O. alutacea var. microspora were previously thought to produce smooth ascospores (Kanouse, 1949; Seaver, 1942; Eckblad, 1968; Dennis, 1978;

Breitenbach and Kränzlin, 1984), but in my transmission and scanning electron microscopic observations, they were all found to produce minutely roughened ascospores covered by mucilagenous-like ridges. The reason why these reticulate ridges are not easily discernible at the light microscopic level is probably due to their more flexible and less cyanophilic nature. Based on similar reasons, Harmaja (1974d) recognized three types of perispores, and he treated Otidea as "temporarily perisporous type" because its cyanophilic perispore-periplasm structure vanishes completely in mature ascospores.

Tarzetta shows another type of spore ontogeny in that secondary wall material is directly deposited on the epispore and is a typical direct precipitation type. Globular bodies present in the epiplasm are often associated with the outer investing membrane and are believed to infiltrate the perisporic sac, depositing secondary wall material, because after spores mature, these kind of bodies are no longer detected. The possible function of globular bodies (or globular structures) was discussed in much detail by Merkus (1975, 1976). There is another type of dense bodies in the sporoplasm which always appears when the translucent wall begins to form and which disappears after the wall is completely constructed. So, these dense bodies are considered another source of wall

material for the inner spore wall formation. A similar case was also found in the Cheilymenia (Chapter II), Mycolachnea, and Trichophaea (Chapter III) where the sporoplasm may donate dense material to the primary wall as it increases in thickness.

Tarzetta is quite different from other species in the Pezizales by its unique pattern of epispore formation. There are no spine-like precursors of the epispore arranged on the primary wall surface before the smooth epispore is formed. Instead, the epispore precursors are deposited directly as periclinal fragments and later on are interconnected into a smooth spore layer (Fig. 4.36).

Cells of paraphyses in Tarzetta are uninucleate, while those of Otidea and most of the Pezizales species have two or more nuclei in both paraphyses and excipular cells (Table 1). Nuclei of Tarzetta always contain condensed chromatin which makes them carminophilic under the light microscope. In another genus with carminophilic nuclei, Leucoscypha, its nuclei also contain extensive condensed chromatin. But Leucoscypha has two nuclei in each paraphysis and excipular cell. The nuclear number and carminophilic reaction are two of the major cytological differences between Tarzetta and Otidea (Harmaja, 1974a; Berthet, 1964; Berthet and Korf, 1969). Additionally, the paraphyses of T. cupularis are easily separated from T.

gaillardiana by their slender crystal inclusion body. This body is visible at the light microscopic level but the composition is uncertain. The difference between the two species of Tarzetta as observed by light microscopic characters has already been discussed by Pant and Tewari (1970).

The third unique difference of Tarzetta which distinguishes it from other species of Pezizales studied thus far is its specific spore wall formation. The "endospore" or primary wall is composed of two subunits, the first formed is an electron dense initial layer and the second is an electron translucent layer. This kind of wall differentiation was also recorded by Schrantz (1966), but was not accepted by Merkus (1975), even though her pictures did show this kind of wall formation.

Table 4.1. Comparison of nuclear number in paraphysis of genera in Humariaceae and Ascobolaceae

Genus	Nuclear Number
Aleuria	2 or more
Ascobolus	2 or more
Cheilymenia	2 or more
Coprobia	2 or more
Leucoscypha	2* or more
Mycolachnea	2 or more
Otidea	2 or more
Scutellinia	2 or more
Tarzetta	1*
Trichophaea	2 or more

*: Nucleus carminophilic.

Figs. 4.1-4.5 Transmission electron micrographs of spore ontogeny in Tarzetta cupularis.

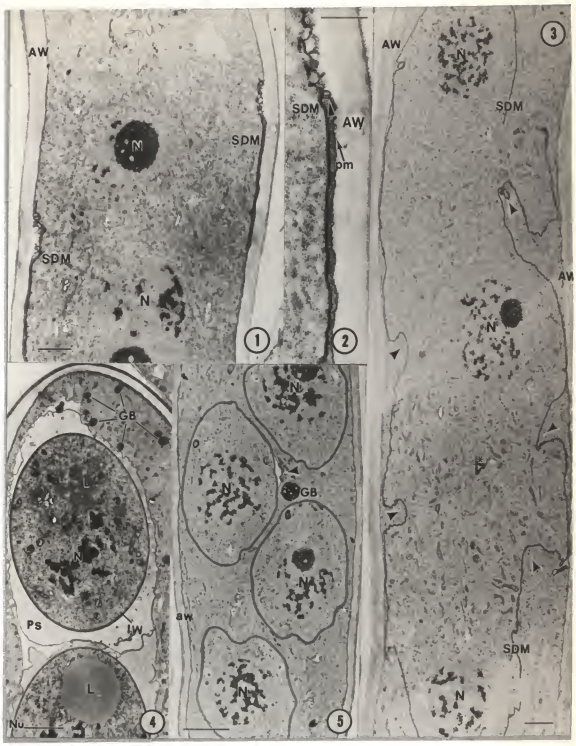
Fig. 4.1 Haploid nuclei (N) and newly differentiated spore delimiting membranes (SDM) (bar = 1 μm). X 11,000.

Fig. 4.2 Detailed view of SDM which is derived from ascal plasma membrane (pm) next to ascal wall (AW) (bar = 0.5 μm). X 30,000.

Fig. 4.3 Haploid nuclei (N) with condensed chromatin and invaginating SDMs (bar = 1 μm). X 9000.

Fig. 4.4 Young ascospores enclosed by electron dense initial wall (IW) and with dilated perisporic sacs (PS). Note the presence of globular bodies (GB) in the epiplasm and smaller lipid droplets (L) inside the sporoplasm (bar = 2 μm). X 7800.

Fig. 4.5 Young ascospores delimited by SDMs within which electron dense initial wall is just deposited. Note the presence of globular body (GB) in the epiplasm and the expanding outer SDM (arrow head) (bar = 2 μm). X 6800.


AW
N
SDM
SDM
N
1
SDM
AW
pm
2
AW
N
3
SDM
AW
N
GB
L
N
Ps
LW
Nu
4
N
GB
N
aw
N
5
SDM
N

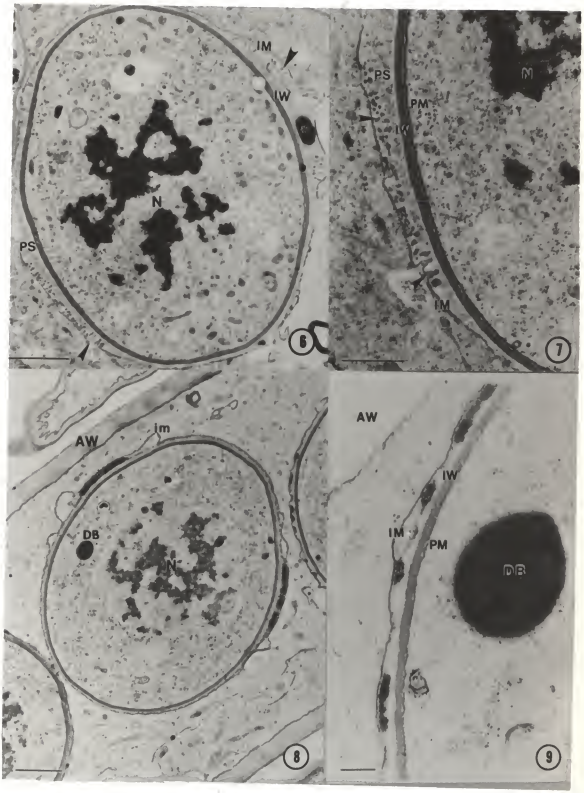
Figs. 4.6-4.9 Transmission electron micrographs of spore ontogeny in Tarzetta cupularis.

Fig. 4.6 A young ascospore enclosed by a thin electron dense initial wall (IW). The outer investing membrane (IM) dilates forming a perisporic sac (PS). Note the presence of electron dense globular wall material inside the PS (asterisk) (bar = 1 μm). X 18,000.

Fig. 4.7 A number of tubular depressions (arrow head) in the investing membrane (IM) (bar = 0.5 μm). X 38,000.

Fig. 4.8 Dense wall material (asterisks) being distributed evenly onto the initial primary wall. Note the presence of dense body (DB) in the sporoplasm (bar = 1 μm). X 14,000.

Fig. 4.9 Dense clumps of wall material (asterisks) interconnected by slim lines (bar = 0.2 μm). X 50,000.


IM
IW
N
PS
6
PS
PM
IW
N
IM
7
AW
im
DB
N
8
AW
IW
IM
PM
DB
9

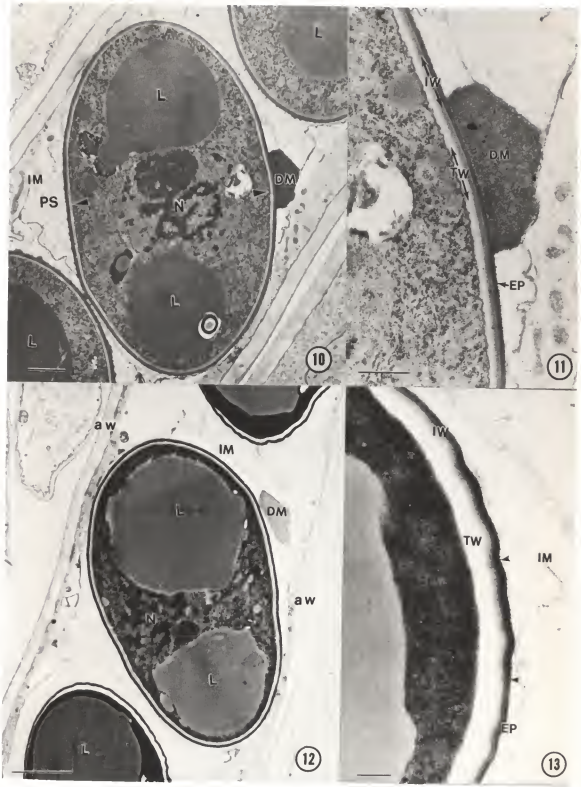
Figs. 4.10-4.13 Transmission electron micrographs of spore ontogeny of Tarzetta cupularis.

Fig. 4.10 A young ascopore includes two large polar guttules (L) and a nucleus with condensed chromatin. Note the dense clump of wall material (DM) attached to the epispore (bar = 1 μm). X 11,000.

Fig. 4.11 A young spore with newly formed translucent wall (TW) under electron dense initial wall (IW). Note a dense clump of wall material (DM) is attached to the epispore (bar = 0.5 μm). X 30,000.

Fig. 4.12 A slightly more mature ascospore with much denser sporoplasm (bar = 2 μm). X 9,000.

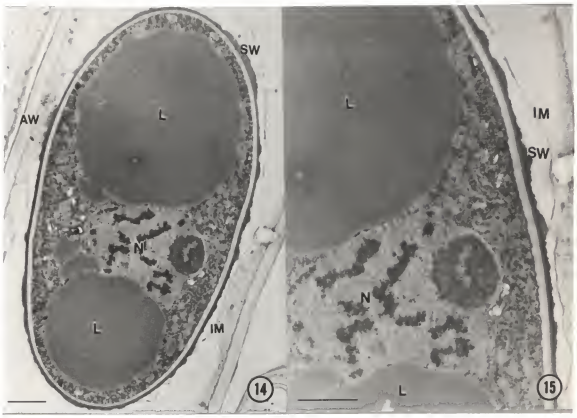
Fig. 4.13 Detailed view of spore wall layers with a thin secondary wall (arrow heads) already deposited on the epispore (bar = 0.2 μm). X 50,000.


L
L
IM
PS
N
DM
L
L
10
IW
TW
DM
EP
11
aw
IM
DM
L
N
aw
L
L
12
IW
TW
IM
EP
13

Figs. 4.14-4.15 Transmission electron micrographs of spore ontogeny in Tarzetta cupularis.

Fig. 4.14 A mature ascospore shown minutely ornamented spore surface (bar = 1 μm). X 11,000.

Fig. 4.15 Detailed view of spore wall layers with undulated secondary wall (SW) covering the spore surface (bar = 1 μm). X 18,000.


AW
SW
L
N
L
IM
14
L
IM
SW
N
L
15

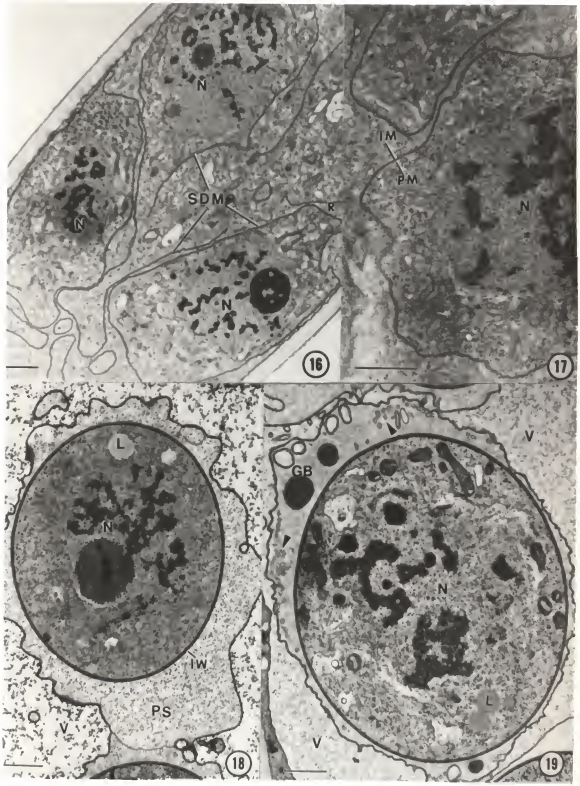
Figs. 4.16-4.19 Transmission electron micrographs of spore ontogeny in Tarzetta gaillardiana.

Fig. 4.16 A young ascus with haploid nuclei which are enclosed by invaginating spore delimiting membranes (SDM) (standard bar = 1 μm). X 10,000.

Fig. 4.17 A haploid nucleus enclosed by an outer investing (IM) and inner sporoplasma (PM) membranes (bar = 1 μm). X 18,000.

Fig. 4.18 A young ascospore enclosed by electron dense initial wall (IW) and surrounded by highly dilated perisporic sac (PS) (bar = 1 μm). X 12,000.

Fig. 4.19 A few dense globular bodies (GB) are present in the perisporic sac. Note the inclusion of small lipid droplets in the sporoplasm (bar = 1 μm). X 12,000.


N
SDM
N
N
16
IM
PM
N
17
L
N
IW
PS
V
18
GB
V
N
L
V
19

Figs. 4.20-4.24 Transmission electron micrographs of spore ontogeny in Tarzetta gaillardiana.

Fig. 4.20 Secondary wall material condensed around the initial wall (IW) forming epispore presursors (EPP) (bar = 0.5 μm). X 35,000.

Fig. 4.21 A young ascospore enclosed by periclinal fragments of epispore precursors (EPP) (bar = 1 μm). X 14,000.

Fig. 4.22 Ascospore is surrounded by electron dense globular bodies (GB) which appear to infiltrate the perisporic sac. Note the presence of dense bodies (DB) in the sporoplasm (bar = 2 μm). X 7500.

Fig. 4.23 A translucent wall (TW) is formed under the electron dense initial wall (IW). A thin layer of secondary wall material is deposited on the epispore (EP) (bar = 0.5 μm). X 7,000.

Fig. 4.24 Expanded perisporic sac (PS) in which a lump of secondary wall material is deposited (asterisk). Note laminated epispore layer (EP) outside the initial wall (IW) (bar = 0.5 μm). X 24,000.

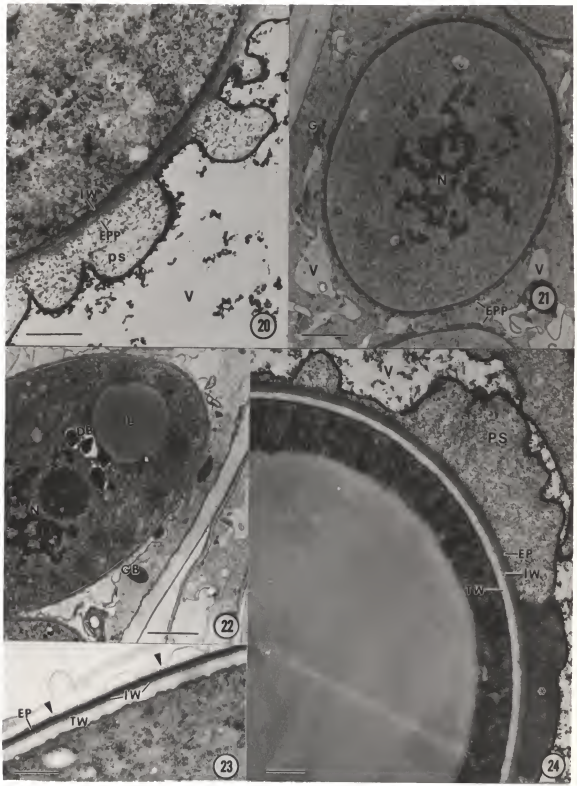
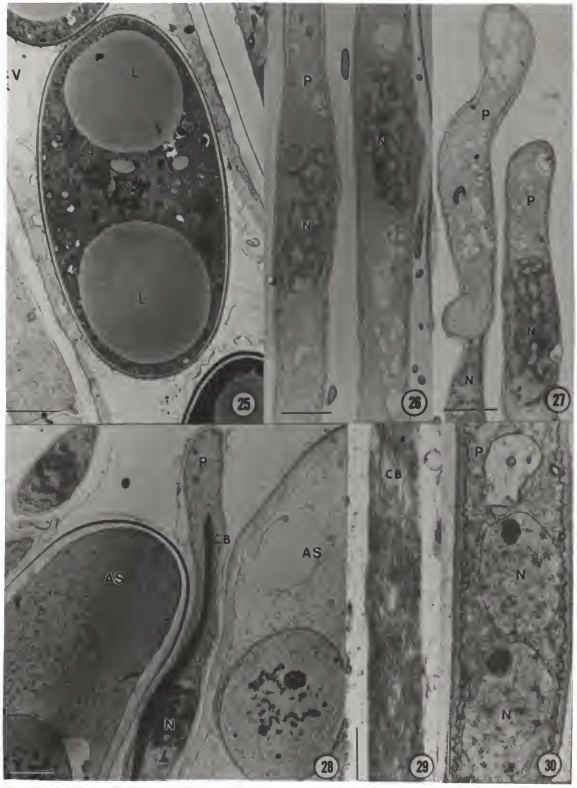

IW
EPP
PS
V
20
C
N
V
V
EPP
21
DB
L
N
GB
22
V
PS
EP
IW
IW
EP
TW
IW
23
24

Fig. 4.25	Transmission electron micrographs of spore ontogeny in Tarzetta gaillardiana.
Fig. 4.25	A mature ascospore with two polar guttules (bar = 2 μm). X 8000.
Figs. 4.26-4.29	Transmission electron micrographs of nucleus in paraphyses of Tarzetta cupularis and Tarzetta gaillardiana.
Fig. 4.26	Uninucleate paraphses in the basal cells of T. gaillardiana (bar = 2 μm). X 8000.
Fig. 4.27	Uninucleate paraphysis tip in T. gaillardiana (bar = 2 μm). X 8,000.
Fig. 4.28	Uninucleate paraphysis (P) between two asci (As) of T. cupularis containing one crystal body (CB) (bar = 2 μm). X6500.
Fig. 4.29	Crystal body (CB) of T. cupularis showing crystal-like lattice feature (bar = 0.5 μm). X 33,000.
Fig. 4.30	Transmission of electron micrograph of nuclei in paraphysis of Otidea alutacea var. microspora.
Fig. 4.30	Two nuclei are observed in the paraphysis (bar = 1 μm). X 16,500.


V
L
L
25
P
N
N
26
P
P
N
N
27
AS
P
CB
AS
N
28
CB
29
P
N
N
30

Figs. 4.31-4.35 Transmission and scanning electron micrographs of species of Otidea.

Fig. 4.31 Spore surface of O. alutacea var. microspora covered by reticulte ridges (bar = 4.29 μm). X 7000.

Fig. 4.32 Reticulate ridges are more concentrated to two polar ends of ascospores in O. onotica (arrow heads) (bar = 12 μm). X 2500.

Fig. 4.33 Reticulate ridges are composed of fibrillar secondary wall material, not firmly consolidated in O. onotica (bar = 0.5 μm). X 42,000.

Fig. 4.34 Spore of O. grandis is covered by reticulate ridges which are more concentrated at polar ends (arrow heads) (bar = 3.75 μm). X 8000.

Fig. 4.35 Smooth ascospores of O. leporina covered by membraneous spore sheath (arrow head) (bar = 6 μm). X 5000.

31
001654 10.0kV X7.00K 4.29µm
32
33
SW
EP
PW
34
001782 4.0 kV X8.00K 3.75µm
35
001771 8.0 kV X5.00K 6.00µm

Fig. 4.36 Comparative spore ontogeny between Tarzetta (A) and Otidea (B and C)

a: outer investing membrane.

b: inner sporoplasma membrane.

c: electron dense initial primary wall.

d: perisporic sac.

e: electron translucent primary wall.

f: epispore precursor.

g: epispore.

h: translucent primary wall.

i: condensed granule.

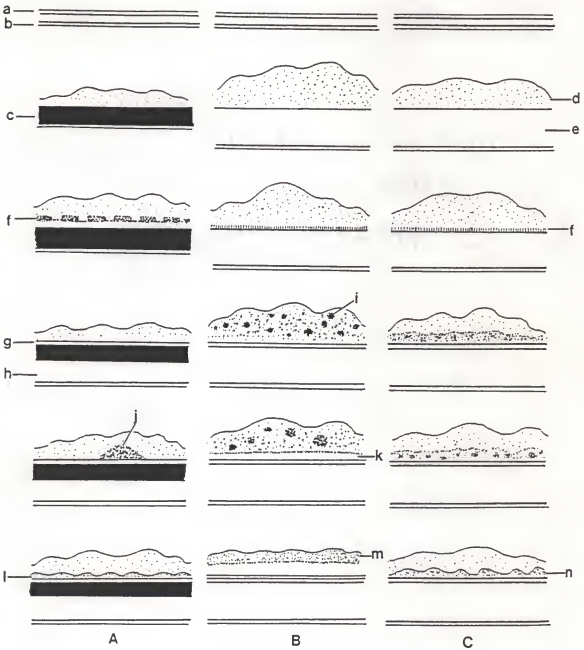
j: secondary wall material.

k: translucent zone.

l: secondary wall material.

m: degenerated perisporic sac (spore sheath).

n: fibrillar secondary wall



36

CHAPTER V
SEPTAL STRUCTURES IN SELECTED GENERA OF ALEURIEAE, LACHNEAE AND OTIDEAE

Introduction

Humariaceae (*sensu* Rifai, 1968) is a heterogenous group of operculate discomycetes producing uninucleate, hyaline ascospores. Since there is a continuous pattern of variation in several apothecial characters, a proper circumscription of this complex taxon into natural and phylogenetically related groups has traditionally been a puzzling problem to mycologists (Samuelson, 1977). Much effort has been made to search for new characters such as ultrastructural features of the operculum and its dehiscence mechanism. Unfortunately, these characters alone can not adequately solve the problem. The so called "Aleuria-Otidea complex" of genera still can not be separated successfully (Brummelen, 1981; Samuelson, 1977).

The tribe Aleurieae mainly differs from Lachneae, in the traditional taxonomy of Humariaceae (*sensu* Rifai, 1968) by their carotenoid pigments and the green reaction of paraphyses with iodine. The Otideae is segregated from the previous two tribes by their glabrous, non-carotenoid apothecia. Additionally, the Lachneae are characterized by their hairy and non-carotenoid apothecia. This kind of

separation, in certain cases, is very ambiguous and controversal. The weakest point in using these light microscopic characters is inadequate data to establish the connections between other families of Pezizales.

Since 1983, a series of papers were published on septal plugging structures in excipular tissue, ascogenous hyphae and ascal bases in which it was demonstrated that septal plugging was very consistent in genera of Pezizaceae and Ascobolaceae (Curry and Kimbrough, 1983; Kimbrough and Curry, 1985). These septal studies inspired contemporary mycologists to determine whether septal structures can be used to sort out the systematics and phylogeny of this heterogenous group. The basic hypothesis is that in the same family, or other naturally related taxa, members should produce the same type of septal structures, especially in the ascogenous hyphae and ascal bases, if they are inherited conservatively.

Kimbrough and Curry (1986a, 1986b) did some preliminary studies on selected genera of Humariaceae and concluded that the family is a very heterogenous group of fungi, probably of polyphyletic origin. In this study, septal structures of selected genera from the tribes Aleurieae, Lachneae, and Otideae are studied extensively in order to establish if septal plugging is a consistent character. If consistency does exist among septal

structures, this may provide a way to establish natural groups and to correlate with light microscopic features.

Concerning the septal structure of the other tribe Ciliarieae (Humariaceae), it will be discussed in the next chapter with another similar septal type found in Ascobolaceae.

Material and Methods

Most of the specimens were collected from the field. After identification, some parts of the collections were accessioned in the herbarium and others were processed for ultrastructural studies following the procedures described by Curry and Kimbrough (1983).

The following specimens were observed: Aleuria aurantia (Pers.: Hook) Fckl. (FLAS-F53706), Gainesville, Alachua County, Florida; Anthracobia muelleri (Berk.) Rifai (ATCC 32246), culture from L. R. Batra, growing on CMMY medium, 12 hr. light and 12 hr. dark, at 25° C.; Caloscypha fulgens (Per. ex Fr.) Boud. (FLAS-F54564), in white pine gulch, ca. 12 mi. NE of Harvard, Latah Co., Idaho; Octospora euchroa (Karst.) Berthet (unaccessioned); Otidea alutacea (Per.) Massee var. microspora Kanouse (FLAS-F55426), on moist leaves and debris along stream near Rustic Falls Rd., 1/4 mi. beyond Rustic Falls, off Bull Pen Rd., 6 mi. S.E. of Highlands, Macon Co., North Carolina; (unaccessioned), on soil near Priest Lake, Bonner Co.,

Idaho; Otidea onotica (Fr.) Fckl. (FLAS-F52959), on soil, by US 19, 3-4 mi. S. of Sutton, West Virginia; (FLAS-F52921), on soil in mixed hardwoods and conifers in Van Neil Glades National Campground ca. 6 mi. W. of Highlands, Macon Co., North Carolina; Pulvinula convexella (Karst.) Pfister (FLAS-F55444), on soil and among mosses, Gainesville, Alachua County, Florida; Mycolachnea hemisphaerica (Wiggers ex. Fr.) Fckl (FLAS-F54599), on sandy soil among mosses at the base of live oak at the American Bank, NW 13 St. and 18 Ave., Gainesville, Alachua County, Florida; (unaccessioned), on soil, in University of Florida Horticultural Farm, Gainesville, Alachua County, Florida; Sphaerosporella brunnea (Alb. & Schw. ex Fr.) Svrcek & Kubicka (FLAS-F55461), on sandy soil, open field in forest near Sugarfoot Apt., Gainesville, Alachua County, Florida; on soil, E. side of Newnan's Lake, Gainesville, Alachua County, Florida; Trichophaea abundans (Karst.) Boud. (FLAS-F55636), on burnt ground, in Dead Lake St. Recreation Area, 1 mi. N. of Wewahitchka, E. of S.R. 71, 5 mi., Florida; T. paludosa Boud. (FLAS-F55637), on soil bank by walkway to sinkhole, in Devil's Millhopper St. Park, Gainesville, Alachua County, Florida.

Plastic blocks were sectioned on an LKB Huxley ultramicrotome with a diamond knife. After poststaining

with uranyl acetate and lead citrate, the sections were examined at 60 kv, on a JOEL 100-CX electron microscope.

Results

Aleurieae

Aleuria aurantia:

Ascogenous hypha- Septal pores are plugged by an electron opaque pulley shaped matrix in which there is an electron translucent and laminated band bordering the pore margin (Figs. 5.1-5.2). The pore diameter is approximately 0.2 μm.

Ascal base- Only half of the pulley-shaped plugging matrix is left in the ascal cell. The laminated band is clearly identified by the pore margin (Fig. 5.3). The pore diameter in this location is 0.3 μm (Table 5.1).

Octospora euchroa:

Ascogenous hypha- A typical pulley-shaped plugging matrix is formed in which a double translucent zone is always associated with the pore margin. When ascogenous hyphae mature and are differentiated into ascal cells, the pore plugging matrix will be pushed toward the direction of the ascal cell and finally form a hemispherical band (Fig. 5.28 E). The pore size of ascogenous hypha is 0.17 μm.

Ascal base- This species shows another different type of septal structure. An electron dense hemispherical band is deposited over the pore of ascal base. Before the ascal pore plugging is fully differentiated, some plugging material is still visible and distributed around the pore region (Fig. 5.4). By the pore border, a double electron translucent zone is present in the plugging matrix (Fig. 5.4, arrow heads). The pore size in the mature ascal base is around 0.17 μm diameter.

Anthracobia muelleri and Caloscypha fulgens

The septal structures of ascogenous hypha and ascal base are exactly the same as found in Octospora euchroa and the pore sizes are about the same, 0.18 μm for A. muelleri and 0.15 μm for C. fulgens (Table 5.1).

Pulvinula convexella

Ascogenous hypha- The electron dense pore plugging matrix is accumulated as a pulley in which a number of electron translucent bands are discernible and some of them are connected into a "V" shape configuration (Fig. 5.5). When ascogenous hyphae develop into asci, the other half of the pulley of plugging matrix disappears and the counterpart in the ascal cell is left (Fig. 5.6).

Ascal base- Alternating dark and white bands in the plugging matrix are arranged parallel from the pore margin to the matrix periphery (Fig. 5.7). Pore diameter is the same as found in the ascogenous hypha, 0.2 μm.

Lachneae

Sphaerosporella brunnea

Ascogenous hypha- A pulley shaped plugging matrix is also found occluding the pore of young ascal cells (Figs. 5.8-5.9) and ascogenous hypha (Fig. 5.10). When ascogenous hyphae become young ascal cells, more matrix will be pushed through the pore and deposited in the ascus side. There is a double translucent torus by the borders of septal pores (Figs. 5.9-5.10, arrows).

Ascal base- An electron opaque hemispherical band is formed over the ascal pore (Figs. 5.11-5.13). The electron density of the hemispherical band increased as the ascus reached maturity (compared with Figs. 5.11 and 5.13). The pore size in the ascogenous hypha and the ascal cell is about the same, 0.15 μm.

Paraphyses- The laminated septal structures (Fig. 5.14, arrow head) in paraphyses are typically surrounded by Woronin bodies.

Mycolachnea hemisphaerica

Ascogenous hypha- A regular pulley-shaped plugging matrix with a double electron translucent band is found in the ascogenous hypha and young ascal cells (Figs. 5.16-5.17). The inner band is striated (Fig. 5.17, arrow heads). In some cases, more than two translucent bands are visible within the matrix in the pore of ascogenous hypha (Fig. 5.17). The pore diameter is 0.2 μm.

Ascal base- When the ascal cell matures, more plugging matrix is pushed toward the ascal cell and finally half of the plugging matrix is left in the inner side of the ascus (Figs. 5.15, 5.18). The pore diameter at the ascal base is measured as 0.3 μm.

Paraphyses/Excipulum- Septal structures are the same in cells of both paraphyses and excipula. A typically laminated structure is found consistently in the plugging matrix and surrounded by a few globular Woronin bodies (Fig. 5.20). In some cases, instead of Woronin bodies, some lipid bodies are observed in the periphery of pore plugging structure (Fig. 5.19).

Trichophaea abundans and T. paludosa

Ascogenous hypha- The pulley shaped pore plugging matrix is the same as found in the other species studied (Figs. 5.21 and 5.23). There is a laminated translucent zone formed by

the septal pore margin separating the plugging matrix (Figs. 5.21-5.22, 5.23, arrows). The pore diameter of both species is 0.2 μm.

Ascal base- In the mature ascal base, a plugging matrix is only found in the ascal cell and separated from pore margin by a laminated translucent zone (Figs. 5.24-5.26, arrow heads). The plugging matrix of T. abundans is a hemispherical mass and shows reticulate features in a mature ascal base. There is a dark band at the bottom of the plugging matrix. However, the plugging matrix of T. paludosa is amorphous and pulley-shaped (Fig. 5.24). The pore size of these two species are different, the former is 0.2 μm and the latter is 0.3 μm.

Paraphyses- The laminated structures are not seen in all pores, but Woronin bodies are always associated with pores. In the paraphyses of T. paludosa, there are some globular dense bodies usually found enclosed in vacuoles (Fig. 5.27).

Otidea alutacea var. microspora and O. onotica

Both of the pore plugging structures of ascogenous hypha and ascal base in O. alutacea var. microspora and O. onotica are the same as found in Octospora euchroa (Fig. 5.4). In both structures, a double translucent zones is always found in the plugging matrix. However, in mature

asci the matrix organizes into a zonate, hemispherical plug (Fig. 5.28 E).

Discussion

In this study, there are three different types of septal structures found in Aleurieae, Lachneae and Otideae. They are the aleurioid (Fig. 5.28C), anthracobioid (Fig. 5.28E) and helvelloid types (Fig. 5.28A & 5.28B).

Aleurioid type of septal structure: This type is characterized by its translucent, laminated zone which is embedded in granular, opaque matrix and borders both sides of the pore in ascogenous hyphae. Later on, the matrix appears to flow into ascal cell, leaving a fan-shaped septal plugging structure at the base of the ascus. This type of septum has been found in the Aleuria aurantia (Kimbrough and Curry, 1986b), Leucoscypha hetieri (Boud.) Rifai (Kimbrough and Curry, 1986b), Mycolachnea hemisphaerica, Trichophaea abundans, and T. paludosa. The pore size of ascal base (0.3 μm) in this type is typically larger in diameter than that of ascogenous hypha (0.2 μm).

This septal type was first described from A. aurantia and A. cestrica (Ell. & Ever.) Seav. by Kimbrough and Curry (1986b). The similarity of septal plugs in ascogenous hyphae and ascus bases was already commented upon, although the sequential connection in the septal development was not

suggested. My observation agrees with Kimbrough and Curry's (1986b) conclusion that Leucoscypha should be placed in this group, but disagrees with their statement that Woronin-like bodies are found in the ascogenous hyphal pores. Although Mycolachnea is temporarily placed in this group, its double translucent zone with an inner striated band is obviously a transitional character between the anthracobioid and aleurioid types.

Anthracobioid type of septal structure: This septal type is characterized by double translucent bands in the opaque plugging matrix of the ascogenous hyphae or crozier. Thereafter, the opaque matrix is pushed through the pore and forms a hemispherical convexed band inside the ascus which is differentiated into two zones, an inner dense band and an outer less opaque zone. In the mature ascal base, the outer zone becomes more condensed and the electron density of inner band becomes into less opaque. There are some electron translucent traces detected in the mature ascal base. This type of septal plugging has been found in Acervus episparticus (Berk. & Br.) Pfister (Kimbrough and Curry, 1986a), A. melaloma (Albt. & Schw.:Fr.) Boud. (Kimbrough and Curry, 1986b), A. muelleri (Kimbrough and Curry, 1986b), C. fulgens , Oc. euchroa (Kimbrough and Curry, 1986b), Ot. onotica, Ot. alutacea var. microspora,

S. brunnea. Many of these species are pyrophilic. The pore size of this type is consistent in both ascal base and ascogenous hypha, although the pore size may be variable from 0.13 to 0.18 μm (Table 5.1).

Both septal structures in ascogenous hyphae and ascus bases have been described (Kimbrough and Curry, 1986b); nevertheless, the sequential development of this septal type and its distinguishing difference from ascoboloid and scutellinioid types (Chapter VI) were not previously noted. The ascus septal structures between aleurioid and anthracobioid types are somewhat similiar, but the aleurioid setal type is fan-shaped, often with an irregular margin. The anthracobioid septal structure is a hemispherical convex band whose margin is very well defined and usually enclosed by a membraneous sheath. Besides, the anthracobioid type is very similar to the hemispherical pore plug found in Ascobolaceae (Chapter VI). Because of the similarities of septal plugs found in the Aleurieae, Kimbrough and Curry (1986b) were inclined to agree with Le Gal's (1953) proposal that Ascobolaceae were derived from Aleurieae.

Caloscypha and Acervus were separated from Aleurieae and treated in Sowerbyelleae (Pyronemataceae) by Korf (1973), because their apothecia discolor on bruising. However, their septal structures and septal development

type are identical as those found in Anthracobia and Octospora which are placed in Aleurieae. Evidently, discoloring reaction of apothecia on bruising is probably not a strong character to be used in separating Sowerbyelleae from Aleurieae.

The two species of Otideae studied showed the same type of septal structures as species of Anthracobia, although the outer morphology of apothecia in Otidea is strikingly different from Anthracobia and other genera showing the same type of septal structures (Table 5.2). We believe that the anthracobioid type of septal structure shows Anthracobia, Caloscypha, Octospora, Otidea and Sphaerosporella to be naturally related, although they were treated in different tribes by Rifai (1968), or subfamilies by Korf (1973).

Blanchard (1972) even described a similar septal structure of ascogenous hyphae with a double translucent torus in the pulley-shaped plugging matrix in Sporormia australis Speg. (Loculoascomycetes) and it is exactly the same as I found in Humariacea (Pezizales). Although he referred this septal structure as similar to that found in Melampsora lini (Ehrenb.) Lev. (Littlefield and Bracker, 1971), the absence of translucent toruses in the septal plug of M. lini suggests they are not identical at all.

The understanding of the relationship between Loculoascomycetes and Pezizales is still very limited.

Helvelloid type of septal structure is only found in Pulvinula convexella. This type has been described in Helvella spp. (Kimbrough and Gibson, 1989), Geopyxis carbonaria (Alb. & Schw.:Pers) Sacc. (Kimbrough and Gibson, 1990), and Gyromitra spp. (Kimbrough, 1991). Although V-shaped striation in the plugging matrix is not prominent in the pore plug of the mature ascus, it does show this feature in the younger ascogenous hypha. The pore size (0.2 μm) is the same in both ascal base and ascogenous hypha (Table 5.1).

In the Aleurieae, three different types of septal structures were discovered. The presence of carotenoid pigment has usually been treated as the major character in Aleurieae (Arpin, 1968; Korf, 1973). Pulvinula produces brightly pigmented apothecia which grow mostly among mosses. However, it shows the same septal type as Geopyxis (Kimbrough and Gibson, 1990) and other helvelloid species (Kimbrough and Gibson, 1989; Kimbrough, 1991) (Fig. 5.28 A, B), even though Pulvinula and Geopyxis, unlike the Helvellaceae, do not produce tetranucleate ascospores. Septal types in these two genera suggest perhaps a

phylogenetic connection between Humariaceae and Helvellaceae.

All of the septal structures found in the excipular and paraphyses cells are laminated structures extending across the pores and associated always with Woronin bodies. This septal type was first described as "Peziza" type by Curry and Kimbrough (1983) and it seems to be a very consistent structure found in the vegetative tissues of apothecia in Pezizales.

This study provides us additional information on septal structures in the Pezizales. If compared with other apothecial characters such as excipular hair, carotenoid pigments, or even habitats, it suggests that septal structures are probably more conservative during fungal evolution. Using this hypothesis, fungi with similar septal structures would be naturally related. The outer structural features such as carotenoid pigments and excipular hairs are considered the results of convergent evolution. Especially, when some fungal species from different origins invade a new ecological niche, they may be destined to evolve certain structures in order to survive or adapt to that niche. Thus, after a number of generations, they may express some similar morphological features, many of which are used by mycologists to arrange

taxonomic groups. The phylogenetic significance of these data will be discussed further in chapter VII.

Table 5.1. Comparison of pore size in the ascal base and ascogenous hypha from selected species of Aleurieae, Lachneae, and Otideae.

Genus name	Ascal base	Ascogenous hypha
*An. muelleri**	0.18 μm[b]	0.18 μm
Al. aurantia	0.3 μm[a]	0.2 μm
C. fulgens	0.15 μm[b]	0.15 μm
*M. hemisphaerica**	0.3 μm[a]	0.2 μm
*Oc. euchroa**	0.17 μm[b]	0.17 μm
Ot. ontica	0.13 μm[b]	0.13 μm
Ot. a. var. *microspora*	0.17 μm[b]	0.17 μm
P. convexella	0.2 μm[c]	0.2 μm
*S. brunnea**	0.15 μm[b]	0.15 μm
*T. abundans**	0.2 μm[a]	0.2 μm
T. paludosa	0.3 μm[a]	0.2 μm

a: aleuroid type; b: anthracobioid type; c: helvelloid type

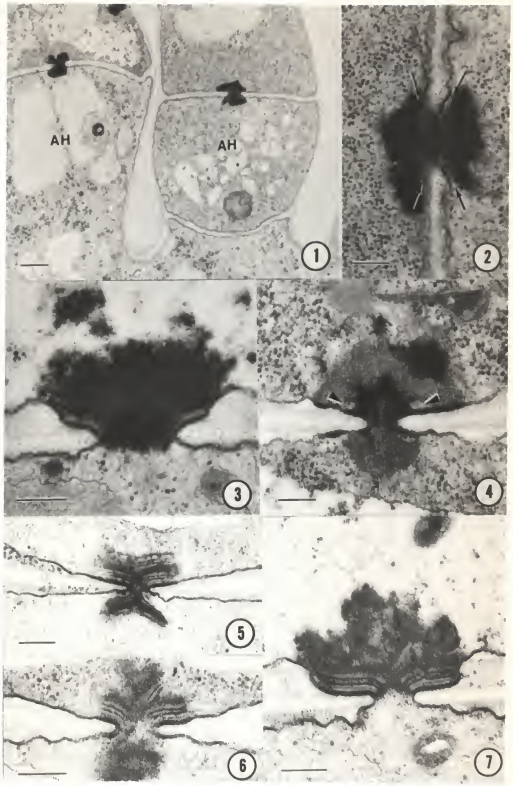
*: pyrophilic species

Table 5.2. A combination of septal structural data and species studied in Rifai's system

Speices	Tribe	Septal type
Al. aurantia	Aleurieae	Aleurioid
M. hemisphaerica	Lachneae	Aleurioid*
T. paludosa	Lachneae	Aleurioid
T. abundans	Lachneae	Aleurioid
An. muelleri	Aleurieae	Anthracobioid
C. fulgens	Aleurieae	Anthracobioid
Oc. euchroa	Aleurieae	Anthracobioid
Ot. ontica	Otideae	Anthracobioid
Ot. a. var. *microspora*	Otideae	Anthracobioid
S. brunnea	Lachneae	Anthracobioid
P. convexella	Aleurieae	Helvelloid

*: with two translucent zones, inner zone with laimination.

Figs. 5.1-7	Transmission electron micrographs of septal structures.
Figs. 5.1-5.3	Aleuria aurantia.
Fig. 5.1.	Ascogenous hyphae (AH) and their pore plugging structures (bar = 1 μm).
Fig. 5.2.	Pore plugging structure of ascogenous hypha showing its striate, translucent zones at the rims of the pore (arrows) (bar = 0.2 μm).
Fig. 5.3.	Pore plugging structure in the mature ascal base showing a granular opaque matrix and striate translucent zone at both sides of the pore (bar = 0.2 μm).
Fig. 5.4	Octospora euchroa.
Fig. 5.4	Hemispherical convex bands at the ascal base showing a translucent trace (arrow heads) at the border of the pore (bar = 0.2 μm).
Figs. 5.5-5.7	Pulvinula convexella.
Fig. 5.5	Septal structure of ascogenous hypha showing multiple striations in which some are V-shaped (bar = 0.2 μm).
Fig. 5.6	Septal structure in a younger ascal base showing multiple striations only in the ascal cell and the other part disappearing (bar = 0.2 μm).
Fig. 5.7	Septal structure in a mature ascal base (bar = 0.2 μm).


AH
AH
1
2
3
4
5
6
7

Figs. 5.8-5.14 Transmission electron micrographs of septal structures of Sphaerosporella brunnea.

Fig. 5.8 A pore plug in a young ascal cell with a diploid nucleus (Nu) and two lipid bodies (L) (bar = 1 μm).

Fig. 5.9 Detailed view of septal structure in young ascal base showing two translucent bands bordering the rims of the pore (arrows) (bar = 0.2 μm).

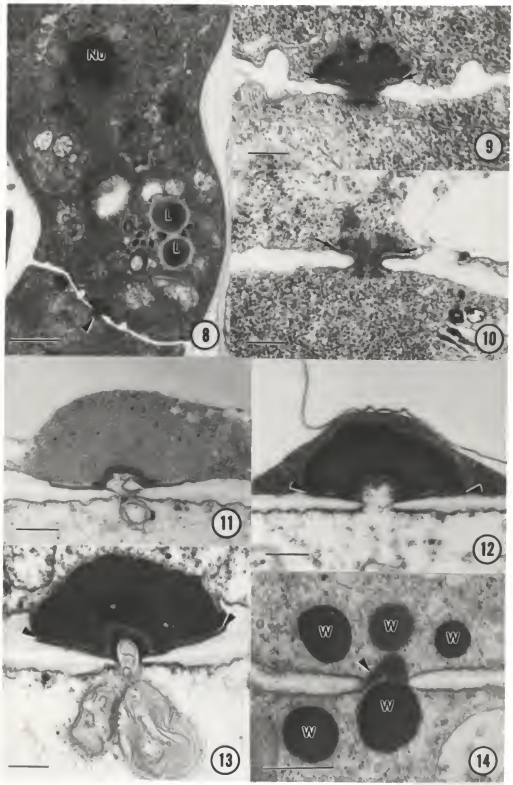
Fig. 5.10 A similar structure found in the septum of ascogenous hypha (bar = 0.2 μm).

Fig. 5.11 An early stage of septal plugging at the base of a mature ascus base. Note the outer part of the hemispherical fan is stained lighter than the inner band (bar = 0.2 μm).

Fig. 5.12 The outer part of hemispherical fan becomes more electron-dense than the inner band. Note a trace of the translucent zone at the base of the hemispherical fan (arrow heads) (bar = 0.2 μm).

Fig. 5.13 A fully developed septal structure at the ascal base (bar = 0.2 μm).

Fig. 5.14 Septal plugging in the paraphysis showing some Woronin bodies and striate structure (arrow head) (bar = 0.5 μm).


Nu
L
L
8
9
10
11
12
W
W
W
W
W
13
14

Figs. 5.15-5.20 Transmission electron micrographs of septal structures of Mycolachnea hemispherica.

Fig. 5.15 Two septal plugs and some lipid globules (L) at the ascal base (arrow heads) (bar = 2 μm).

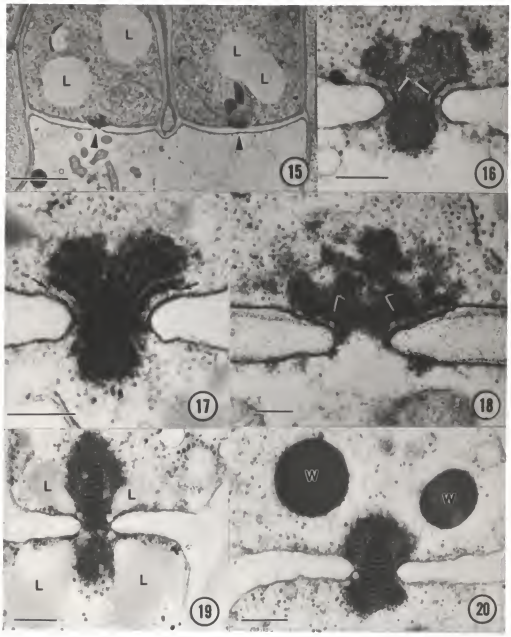
Fig. 5.16 A septal structure in the ascogenous hypha showing double translucent bands bordering the pore. Note the outer band is not striated (arrow heads) (bar = 0.2 μm).

Fig. 5.17 A detailed view of a septal structure in the ascogenous hypha with an inner striated and translucent band (arrows) (bar = 0.2 μm).

Fig. 5.18 A septal plug at the base of a mature ascus. Only half of the plug is shown inside the ascal cell (bar = 0.2 μm).

Fig. 5.19 An excipular septum is characterized by a striated structure inside the opaque matrix which pluggs the pore. Note the presence of lipid drops associated with pore plugging (bar = 0.2 μm).

Fig. 5.20 A paraphysis septum showing a similar striated structure and Woronin bodies with excipular cells (bar = 0.2 μm).


L
L
L
L
15
16
17
18
L
L
L
L
19
W
W
20

Figs. 5.21-5.27 Transmission electron micrographs of septal structures of Trichophaea.

Fig. 5.21 Septal plugging in the ascogenous hypha of T. abundans (bar = 1 μm).

Fig. 5.22 Striated translucent zones (arrows) shown in the septal plug of a young ascal base of T. abundans (bar = 0.2 μm).

Fig. 5.23 A septal structure in the ascogenous hypha with a translucent striated band around the rim of pore in T. paludosa (bar = 0.2 μm).

Fig. 5.24 A septal structure with a laminated zone in the ascal base showing half of the pore plug inside the ascal cell in T. paludosa (bar = 0.2 μm).

Fig. 5.25 Opaque matrix diffusing through pore into ascal cell and forming a hemispherical fan leaving translucent traces at the base of the plugging structure in T. abundans (bar = 0.2 μm).

Fig. 5.26 A hemispherical opaque fan becoming reticulate in T. abundans (bar = 0.2 μm).

Fig. 5.27 A septal structure in the paraphysis of T. paludosa with its associated Woronin bodies (W) and two membrane-bound dense bodies (DB) (bar = 1 μm).

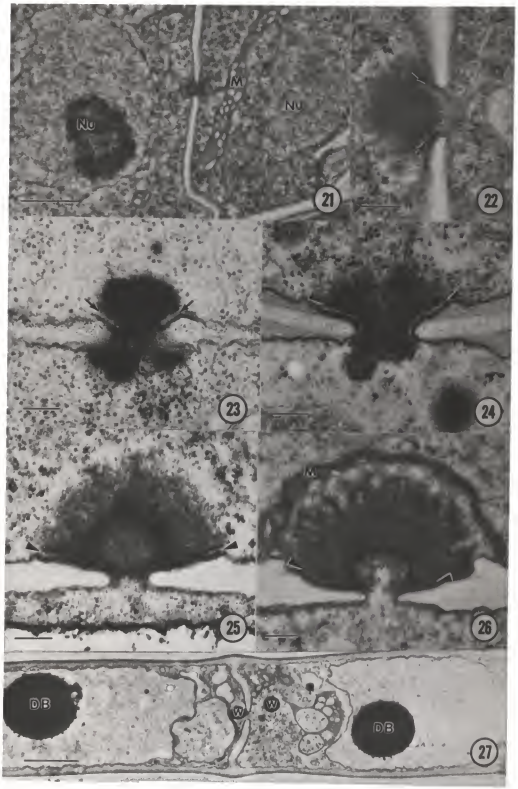

Nc
M
Nu
21
22
23
24
M
25
26
DB
W
W
DB
27

Fig. 5.28 Diagramatic sketches of different developmental stages of septa found in Humariaceae (from left to right).

A. Helvelloid type found in Helvella, Gyromitra, and Geopyxis.

B. Helvelloid type found in Pulvinula.

C. Aleurioid type found in Aleuria and Trichophaea.

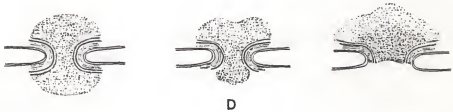
D. Transitional type between aleurioid and anthracobioid types found in Mycolachnea.

E. Anthracobioid type found in Anthracobia, Caloscypha, Octospora, Otidea, and Sphaerosporella

A

B

C



D

E

(28)

CHAPTER VI
COMPARATIVE STUDIES ON THE SEPTAL DEVELOPMENT BETWEEN ASCOBOLOID AND SCUTELLINIOID TYPES

Introduction

Most Ascobolaceae are found on dung, although some of them may grow on charcoal, soil or rotten wood. This ecological habitat matches very well with members of the tribe Ciliarieae (Humariaceae). Most of the Ciliarieae (=Scutellinieae *sensu* Korf, 1973), however, produce hyaline ascospores and hairy apothecia with bright carotenoid pigments. From previous ultrastructural data on ascosporogenesis of Ascobolus (Wu and Kimbrough, 1991a, 1991b), Scutellinia, Cheilymenia, and Coprobia (Chapter II), a very close similarity between Ascobolaceae and Ciliarieae (Humariaceae) has been shown.

A similar conclusion was drawn by Kimbrough and Curry (1986) who studied the septal structures in the Scutellinieae and Sowerbyelleae tribes of Pyronemataceae (*sensu* Korf, 1973) (=Humariaceae *sensu* Rifai, 1968) and pointed to a close relationship of Scutellinieae (=Ciliarieae, Rifai 1968) to the Ascobolaceae.

Even though both septal structures and spore ontogeny types are shown to be very similar in the Ciliarieae and Ascobolaceae, the morphological distinction between the two

groups is still very prominent. Whether these two groups should belong to the same family, or whether they evolved from the same ancestor, could be confirmed from developmental, cytological and ultrastructural studies. If enough characters correlate, they may be placed in the same family, or they may prove have only a close phylogenetic linkage.

The main purpose of this paper is to compare septal development in species of the Ciliarieae (=Scutellinieae, Pyronemataceae *sensu* Korf, 1973) with that of Ascobolaceae and to establish possible relationships among these groups.

Material and Methods

Field collections were processed for transmission electron microscopy following the procedures described by Curry and Kimbrough (1983). The following specimens were observed: Ascobolus stictoideus Speg. (FLAS-F53512), on goat dung from Araya, Sucre State, Venezuela; Cheilymenia coprinaria (Cke.) Boud. (FLAS-F53436), on cow dung, SW 20th Av., near I-75, Gainesville, Alachua County, Florida; C. stercorea (Pers. ex Fr.) Boud. (FLAS-F53435), on cow dung, SW 20th Ave., near I-75, Gainesville, Alachua County, Florida; Coprobia granulata (Bull. ex Mérat) Boud. (FLAS-F55143), on cow dung, Sugarfoot Hammock, on south side of SW 20th Ave., ca. 0.5 mi. W. of I-75, Gainesville, Alachua County, Florida; Scutellinia scutellata (L. ex Fr.)

Lambotte (FLAS-F55434), on wood stump covered with mosses, behind Art Center, Highlands Biological station, Highland, North Carolina.

Plastic blocks were sectioned on an LKB Huxley ultramicrotome with a diamond knife. After poststaining with uranyl acetate and lead citrate, the sections were examined at 60 kv, on a JEOL 100-CX electron microscope. In order to observe inner structures inside the septal plugging matrix, some of sections, before examined by electron microscope, were treated by 1% hydrogen peroxide for 1-5 minutes following regular poststaining precedure.

Results

Ascobolus stictoideus: Septal structures in the crozier stage are observed in A. stictoideus (Fig. 6.1). In the central lumen of the pore, there are some delicate vacuoles surrounded by endoplasmic reticulum (Figs. 6.2-6.3, arrow). Subsequently, some less electron opaque material is deposited around the vacuoles (Fig. 6.3, asterisks). More material is accumulated forming arches across the pore (Fig. 6.4, asterisks). In the mature ascus, an electron opaque hemispherical band is formed (Fig. 6.5). Usually, the lower part of hemispherical structure is more condensed and electron opaque than the outer dome, and the opening of the pore is sealed by a membranous bulb.

Scutellinia scutellata: Pore plugging structures in the ascogenous hypha and crozier stages are observed (Fig. 6.6, arrow heads). Electron opaque plugging matrix accumulates in the pore center, but separates from the pore border by a translucent torus (Figs. 6.7-6.9). Thereafter, a number of small vacuoles are formed within the plugging matrix (Figs. 6.9-6.10, arrow head). Vacuoles push the plugging matrix to both sides forming biconvex arches (Figs. 6.10-6.12). The contents of the biconvex arches are removed when they are treated with 1% hydrogen peroxide (Figs. 6.12, 6.14). The lower part (opposite to the ascal cell) is usually diminished gradually (Fig. 6.12, arrow heads). Another layer of less opaque material, very inert to Hydrogen Peroxide (Fig. 6.14), is deposited on the main arch (Fig. 6.11). So, in the mature ascal base, a hemispherical band with two different zones is formed (Figs. 6.13-6.14). A membranous veil is usually found on the mature septal arch (Fig. 6.13, arrow heads). Below the hemispherical band, a poorly formed laminated structure (Fig. 6.15, arrows) is rarely found within the pore.

This type of septal development is also found in species of Cheilymenia and Coprobia. In C. coprinaria and C. stercorea, a similar electron dense arch is formed first at the ascal base and then another less opaque material is deposited, forming a double zonate band (Figs. 6.12-6.20).

However, there is no additional less opaque layer deposited on the electron dense band in Coprobia granulata. In an old ascus, the lower part of the septal structure is occluded by hyphal wall material (Fig. 6.18).

Discussion

This study has shown that three different groups of cup fungi may have a very similar hemispherical banded pore plug at the ascal bases (Fig. 6.21). They are species of Ascobolaceae and Ciliarieae and Anthracobia-Otidea groups of Humariaceae (Chapter V). However, their manner of septal development is totally different and deserve to be recognized individually in order to distinguish them and for convenience in the following discussion. These septal types will be referred to as the ascoboloid (Fig. 6.21 C), scutellinioid (Fig. 6.21 B), and anthracobioid (Fig. 6.21 A) types respectively.

Ascoboloid type of septal structure: This type is characterized by the presence of delicate vacuoles surrounded by endoplasmic reticulum in the central lumen of the septal pore of ascogenous hypha, and the pore plugging material is secreted around the vacuoles. A hemispherical band is finally formed in the mature ascal base. The pore diameter for ascogenous hypha is 0.14-0.27 μm and for the ascal base is a little smaller, 0.2 μm.

This septal development type is found in all members of Ascobolaceae (Wells, 1972; Kimbrough and Curry, 1985). The term "ascoboloid" was first coined by Kimbrough and Curry (1985) to describe a hemispherical structure in the ascal septa of taxa of the Ascobolaceae. Even though they did also observe the septal structures in ascogenous hyphae, however, the developmental connection between ascogenous hypha and ascal base was not emphasized. In this study, I examined carefully the septal structures from the ascogenous hyphae, croziers and ascal bases, and suggested a unique developmental type in the Ascobolaceae.

Scutellinioid type of septal structure: This type is characterized, in the septal structures of ascogenous hyphae, by the presence of a translucent torus by the pore border in the plugging matrix within which delicate vacuoles are visible. When the ascogenous hyphae mature and become young asci, a plugging matrix is pushed toward both sides forming two hemispherical archs. In the base of mature asci, only one hemispherical band is left in the ascal cell on which later another layer of wall material will be deposited. The pore diameter of ascogenous hypha is 0.3-0.36 μm and of ascal base is 0.2-.3 μm.

This septal development type has so far been found only in members of Ciliarieae (Humariaceae). There are two

major differences separating scutellinioid type from ascoboloid type, (i) the presence of a translucent torus by the pore border of ascogenous hyphae, (ii) biconvex arches are formed due to an assumptive pressure created inside the plugging matrix. However, the components of the hemispherical structure of the ascoboloid type are secreted by endoplasmic reticulum and deposited around the vacuoles located within the pore lumen. The hemispherical structure of the scutellinioid type is obviously composed of two different layers. The inner layer is very osmiophilic and perhaps constituted of lipid or lipoprotein which provides very good occlusion capability. These components are removed with the hydrogen peroxide treatment. In contrast to the inner layer, the outer dome is inert to hydrogen peroxide and may be composed of different materials such as polysaccharides which have been suggested to be in the plugging matrix of Helvella and Gyromitra (Kimbrough and Gibson, 1989). Information about the chemical composition of septal structures is very limited thus far.

Kimbrough and Curry (1986a) also studied the septal structures of ascogenous hyphae and ascal bases separately in members of Ciliarieae. A similar hemispherical structure was described in ascal septa and suggested a close relationship to Ascobolaceae. However, the septal structures of ascogenous hyphae were different from that

found in this study. Kimbrough and Curry (1986a) found Woronin bodies were associated with septal plugs of ascogenous hyphae.

It is an unusual phenomenon to find laminated structures within the pores of ascal bases (Fig. 6.15), because these structures were only described from Pezizaceae (Curry and Kimbrough, 1983). This phenomenon and the potential to form a biconvex structure suggest a possibility that Ciliarieae and Pezizaceae might be phylogenetically related.

Anthracobioid type of septal structure: This septal type has been described in the Chapter V and is different from the two other types by the presence of two translucent zones adjacent to the pore borders, and the absence of those delicate vacuoles in the plugging matrix (Fig. 6.21).

This septal development type has been found in species of Anthracobia, Caloscypha, Octospora, Otidea, Pyronema, and Sphaerosporella. The significance of this type of septal structure and phylogenetic relationship with other types have been discussed in Chapter V.

The phylogenetic connection of these three types is not only based on the septal development, but also on spore ontogeny. During spore ontogeny, all three groups of fungi form condensed granules in the perisporic sac that

gradually attach to the epispore forming ornamentations or secondary wall. Additionally, a translucent zone in the perisporic sac is found consistently in some pyrophilic (*i.e.*, Anthracobia, Octospora etc.) and soil-inhabiting species (*i.e.*, Scutellinia etc.) which suggests a strong connection with Ascobolaceae, which have a unique spore ultrastructure (Wu and Kimbrough, 1991a, 1991b, Chapter II, III, and IV).

Species showing the same type of septal structures in the pores of mature asci, but exhibiting transitional differences in their early development stages, point to a possible monophyletic origin of these groups. The study of septal development may be potentially of great use in the study of phylogeny in the Pezizales.

Figs. 6.1-6.7 Transmission electron micrographs showing developements of ascoboloid and scutellinioid septal structures.

Figs. 6.1-6.5 *Ascobolus stictoideus*.

Fig. 6.1 A young crozier (C) showing one of the pore plugs (arrow head) (bar = 2 μm).

Fig. 6.2 A number of delicate vacuoles (arrow) are present in the central pore lumen of ascogenous hypha which is surrounded by endoplasmic reticulum (bar = 0.2 μm).

Fig. 6.3 Some electron opaque material (asterisks) is deposited around the pore (bar = 0.2 μm).

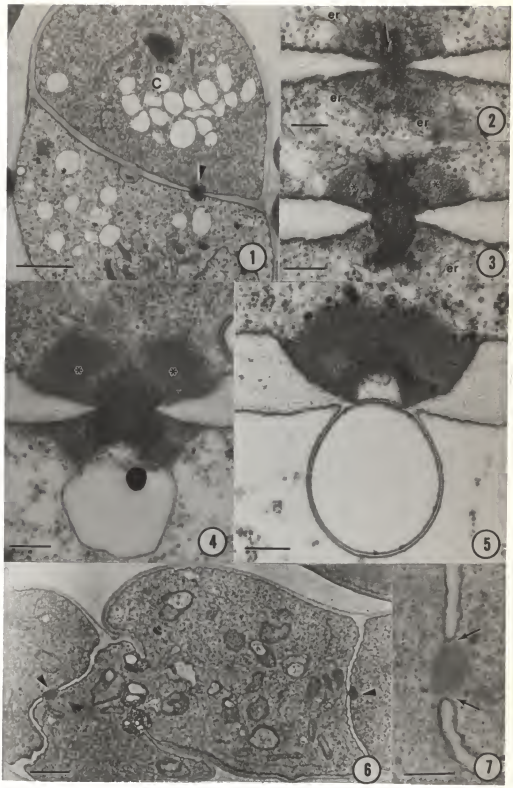
Fig. 6.4 Some electron opaque material (asterisks) is deposited around the pore (bar = 0.2 μm).

Fig. 6.5. An electron opaque, hemispherical band is laid across the ascal pore (bar = 0.2 μm).

Figs. 6.6-6.7 *Scutellinia scutellata*.

Fig. 6.6 Septal pluggings (arrow heads) in a section of ascogenous hypha. Left septal structure is younger than right side (bar = 1 μm).

Fig. 6.7 A translucent zone (arrows) shown in the pore plugging matrix. A higher magnification of septal structure (left) in Fig. 6.6 (bar = 0.5 μm).


C
er
er
er
er
1
2
3
4
5
6
7

Figs. 6.8-6.15 Transmission electron micrographs showing the development of scutellinioid septal structure.

Figs. 6.8-6.15 Scutellinia scutellata.

Fig. 6.8 Pore plugging matrix in ascogenous hypha with a translucent zone (arrows) (bar = 0.2 μm).

Fig. 6.9 Some small vacuoles are present in the central lumen of pore plugging matrix (bar = 0.2 μm).

Fig. 6.10 More vacuoles (arrow head) are present in the pore lumen and biconvex bands are formed (bar = 0.5 μm).

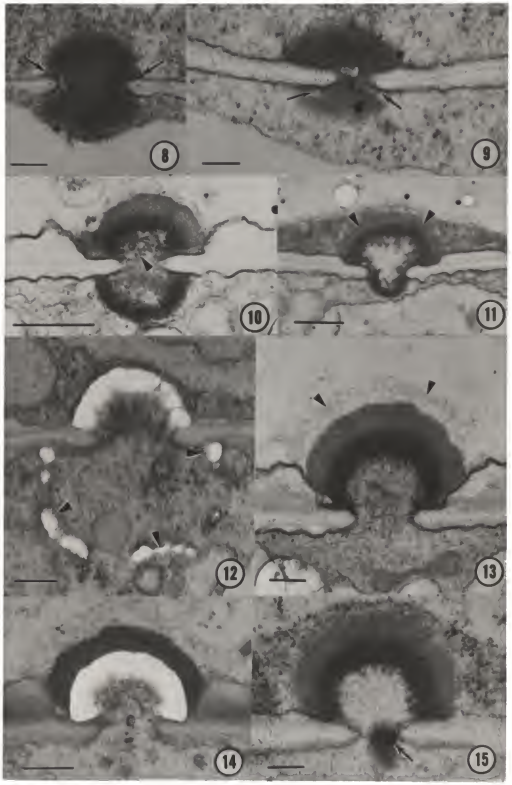
Fig. 6.11 Another less electron opaque layer (arrow heads) is laid over one of the biconvex bands in ascal cell (bar = 0.5 μm).

Fig. 6.12 After bleached by 1 % hydrogen peroxide, the lower part of biconvex bands degenerating (arrow heads) (bar = 0.2 μm).

Fig. 6.13 A hemispherical band in a mature ascal base and the upper part covered by a membranous structure (bar = 0.2 μm).

Fig. 6.14 A hemispherical band was treated by 1 % hydrogen peroxide. The lower part is bleached and formed earlier than upper part (bar = 0.3 μm).

Fig. 6.15 In certain cases, another laminated structure (arrow) is found under the dome-shape plugging band (bar = 0.2 μm).


8
9
10
11
12
13
14
15

Figs. 6.16-6.20 Transmission electron micrographs of septal structures in Coprobia and Cheilymenia.

Figs. 6.16-6.18 Coprobia granulata.

Fig. 6.16 An electron dense hemispherical band at the ascal base (bar = 0.5 μm).

Fig. 6.17 An electron dense hemispherical band at the ascal base (bar = 0.5 μm).

Fig. 6.18 A mature septal plugging at the ascal base and lower part is occluded by hyphal wall material (bar = 0.2 μm).

Figs. 6.19-6.20 Cheilymenia stercorea.

Fig. 6.19 Two early ascal plugging structures at the ascal bases, showing only electron dense hemispherical bands (bar = 1 μm).

Fig. 6.20 A mature ascal plugging structure, showing another less opaque material (arrow heads) laid over electron dense hemispherical band (bar = 0.5 μm).

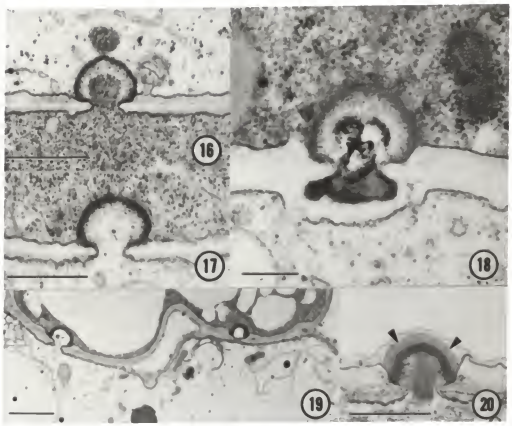

16
17
18
19
20

Fig. 6.21 A comparision of septal developments between anthracobioid (A), scutellinoid (B) and ascoboloid (C) types.

A

B

C

21

CHAPTER VII:
SPORE ONTOGENY, SEPTAL STRUCTURE, CHARACTERS USEFUL IN SHOWING PHYLOGENY IN PEZIZALES

Introduction

Spore ontogeny (Chapters I-IV) and septal structures (Chapter V-VI) have been found to be consistent in the subgroups of Humariaceae (Table 7.1). However, these subgroups recognized by these two new sets of characters are not equal to those proposed previously by Eckblad (1968), Rifai (1968), Arpin (1968), Dennis (1968), or Korf (1973) (compare with Table 7.1 and 7.2). These two sets of characters are thought at this moment to be the most conservative features in the taxonomy of Pezizales, since some of the traditional light microscopic characters such as hymenial colors may be unstable and changed under different environmental conditions or developmental stages (Batra, 1960; Moore and Korf, 1963). Besides, some taxa such as Melastiza and Aleuria are even separated mainly by the presence of excipular hairs or not. Another artificial character used to distinguish Trichophaea from Mycolachnea is by their apothecial forms. All of these characters have been considered having a low taxonomic values (Eckblad, 1968). Septal structure and spore ontogeny do provide a

new insight to the rearrangement of taxa in the Humariaceae.

Descriptions

Spore Ontogeny:

In this study (chapter I-IV), only two general types of spore ontogeny are recognized: "gradual condensation" and "direct precipitation" (Fig. 7.1).

Gradual Condensation Type: After the primary wall is formed between two spore delimiting membranes, the outer investing membrane will dilate forming a perisporic sac within which secondary wall material is deposited. At this stage, the secondary wall material may temporarily appear as dense clumps attached to the primary wall (Fig. 7.1A, e); however, this dense material is broken very soon and distributed evenly within the perisporic sac. Thereafter, radial spine-like precursors of the epispore layer are synthesized from the secondary wall material and arranged on the surface of the primary wall. Later on, a more periclinal connection occurs between radial spines to form smooth epispore layers. Secondary wall material condenses randomly in the perisporic sac into globules or amorphous granules which will gradually coagulate on the epispore, forming ornaments. The genera showing this type of spore ontogeny (GC) are outlined in Table 7.1.

Another modified gradual condensation type is found in the pyrophilic species examined such as Anthracobia (Chapter V), Octospora (Chapter I), Sphaerosporella and Trichophaea (Chapter III), and some coprophilous fungi such as Cheilymenia, Coprobia (Chapter II), Ascobolous (Wu and Kimbrough, 1991a, 1991b, Wells, 1972), and Saccobolus (unpublished data). It is characterized by the presence of a translucent zone between epispore layer and perisporic sac (Fig. 7.1B, i). This translucent zone with a well defined electron dense band is found only in coprophilous fungi. Here, the condensed granules will gradually attach to this dense band forming ornaments. Ornaments do not attach directly to the epispore, but form a loose crust over the "endospore". This perhaps explains why the outer spore wall of Ascobolous, Cheilymenia, and Coprobia is so fragile and is broken easily leaving a hyaline and smooth "endospore" on mounts for light microscopy. However, in pyrophilic species, the translucent zone is not defined as clearly in the perisporic sac. Once differentiation of this zone is complete, the secondary wall material in the perisporic sac starts degenerating, even though the random condensation of secondary wall material is still detected. Finally, the aborted perisporic sac becomes an ephemeral sheath covering the whole smooth "endospore". At maturity,

the "endospore" is only ensheathed by the epispore layer (Figs. 7.1B and 7.2A).

Direct Precipitation Type: Without gradual condensation in the perisporic sac, the secondary wall material condenses directly onto the primary wall surface. From the inner zone of this material, an epispore layer is differentiated. The residues keep condensing and may be transformed into ornaments (Fig. 7.1C). Spore ontogeny in Pulvinula convexella is typical of this type. This species is reported to have smooth ascospores, but it is shown to have tiny rodlets on the surface that leave a fine echinulation. In another speices, Geopyxis carbonaria (Kimbrough and Gibson, 1990), however, the secondary wall material in the perisporic sac will directly condense at the beginning into spinelike precursors of epispore (Fig. 7.2B). After the smooth epispore is formed, more secondary wall material is condensed directly on the epispore surface forming secondary wall.

A third genus in Humariaceae that has been observed with this type of spore ontogeny is Tarzetta (Chapter IV). It differs from Pulvinula and Geopyxis, however by its unusual type of epispore layer formation. Tarzetta does not form spinelike precursors on the primary wall surface, but instead forms a series of periclinal fragments which

are suspended within the perisporic sac and finally settle on the primary wall directly forming an epispore layer.

Therefore, the standard line to distinguish gradual condensation from direct precipitation types is set at the time that formation of the epispore layer is finished. If the secondary wall material is condensed into granules, after the epispore formed, no matter if it is globular or amorphous, it is placed in the gradual condensation type. In contrast to the gradual condensation type, there are no condensed granules present in the perisporic sac after the formation of epispore.

In Mycolachnea hemisphaerica and Trichophaea paludosa, a transitional pattern between "gradual condensation" and "direct precipitation" types was found (Chapter III). In these species, secondary wall material is deposited closer to the primary wall and from this material an epispore layer is differentiated. Other residual wall material then gradually condenses into granules which later will attach to the epispore and form the ornaments. If this secondary wall material is deposited very compacted, and closer to the primary wall and without the formation of condense granules, the spore ontogeny will be similar to the direct precipitation type (Fig. 7.3).

Septal Structure

Four contrastingly different developmental types of septal structures in ascogenous hyphae and ascus bases are found in genera of Humariaceae, bringing to seven the different types in the families of Pezizales (Table 7.1).

1. Ascoboloid Type: In the ascogenous hyphae and crozier, some vacuoles are found in the central pore lumen and are surrounded by ER. Then, in the younger ascal base, some electron-dense material is deposited around the septum, forming a zonate hemispherical band (Fig. 7.4, A1 & B1) (Kimbrough and Curry, 1985).

2. Scutellinioid Type: A translucent zone is first formed in the plugging matrix. Subsequently, some vacuoles arise in the center and push the plugging matrix to both sides. Within the ascus a hemispherical band forms on which another layer is laid down as the ascus matures (Fig. 7.4, A2 & B1).

3. Anthracobioid Type: This type is characterized by a double translucent zone in the pores of ascogenous hyphae and croziers. As a greater amount of cytoplasm flows into the ascal cell, a hemispherical band is formed that looks similar to the ascoboloid type (Fig. 7.4, A3 & B1).

4. Aleurioid Type: In this type, a laminated translucent torus forms in the pores of ascogenous hyphae. When the granular matrix flows through the pore, a somewhat

hemispherical mass of plugging material is left in the ascal base (Fig. 7.4, A5 & B3) (Kimbrough and Curry, 1986a). The transitional stage between type three, four and five is found in the Mycolachnea hemisphaerica. It produces double translucent zones, but the inner band is laminated (Fig. 7.4, A4 & B2).

5. Helvelloid Type: In the ascogenous hyphae, numerous translucent bands appear in the plugging matrix. In the mature ascal base, however, only V-shaped translucent bands are shown (Fig. 7.5 G) (Kimbrough and Gibson, 1990; Kimbrough, 1991). Kimbrough (1990) found an almost identical type of ascus pore plug in taxa of Morchellaceae (Fig. 7.5 H). Pulvinula convexella exhibits an important transitional pattern in Humariaceae between the helvelloid and aleurioid types (Fig. 7.4, A6 & B4).

6. Pezizoid Type: This type found in all genera of Pezizaceae is characterized by the production of electron-opaque biconvex bands at the ascal bases (Schrantz, 1970; Pepin, 1971; Curry and Kimbrough, 1983).

7. Ascodesmioid Type: This type is specialized by producing a hemispherical band on which there is an array of radiate tubercular structures (Carroll, 1966; Brummelen, 1989).

Both pezizoid and ascodesmioid septal developmental types have not been adequately studied yet, even though

their septal structures have been observed and well documented (Curry and Kimbrough, 1983; Carroll, 1966; Brummelen, 1989). Studies on septal plug ontogeny in Morchellaceae are not complete, but preliminary observations show a type of septal structure close to the helvelloid type (Kimbrough, 1990).

An alternating white and dark laminated structure extending across the pores of excipular and paraphysis cells is found very consistently in all of the species of Pezizales studied thus far (Schrantz, 1964; Curry and Kimbroug, 1983; Janex-Favre *et al.*, 1988; Kimbrough and Curry, 1985, 1986a, 1986b; Kimbrough and Gibson, 1989; Kimbrough, 1991), and this lamellate structure has been named the "Peziza" type (Curry and Kimbrough, 1983) in order to distinguish it from the "Neurospora" septal type found in other groups of ascomycetes (Camp, 1977; Benny and Samuelson, 1980; Reichle and Alexander, 1965; Cole and Aldrich, 1971; Hammill, 1974; Bronchart and Demoulin, 1975).

Discussion

All of the genera of Pezizales studied thus far can be separated into two groups based on spore ontogeny: (i) one produces spore walls by "gradual condensation type", and (ii) the other by "direct precipitation type". A transitional stage, or intermediate type, is present in

Mycolachnea hemisphaerica and Trichophaea paludosa. Coincidently, the septal structure of Mycochlachnea is also a transitional pattern between the helvelloid and aleurioid types (Fig. 7.4). It is unique to find that distinct spore ontogeny types always correlate with specific types of septal structures (Table 7.1). In all of the genera with the gradual condensation type of spore ontogeny, their septal structures are similar to the ascoboloid type. While those with the direct precipitation type of spore wall ontogeny have other types of septal plug configuration. Five septal types, the ascoboloid, scutellinioid, anthracobioid, and aleurioid, including the transitional type in Mycolachnea show the same type of spore ontogeny (Fig. 7.3). Especially in the ascoboloid, scutellinioid and anthracobioid types, they all have a translucent zone differentiated in the perisporic sac. The hemispherical bands in pore plugs of mature asci are also exactly same, even though their developmental patterns are somewhat different. This suggests that the species with these septal and spore ontogeny types have probably evolved from a common ancestor.

From the developmental study of septal structures, Humariaceae is proving to be an evolutionarily central group which connects very well with the families Ascobolaceae, Pezizaceae, and Helvellaceae (Figs. 7.4-7.5).

A sequential change is evident from the ascoboloid to the helvelloid type, no matter if the septal structures are in the ascogenous hyphae or ascal bases. From the ascogenous hyphae, septal plugging in the ascoboloid type is initiated by the deposition of dense material around the central vacuolate region by the endoplasmic reticulum. This is followed then by the scutellinioid type which is specialized by the presence of a translucent zone by the border of the pore and the appearance of vacuoles in the central plugging matrix. These vacuoles are believed to be an evolutionary trace from the ascoboloid type. Double translucent zones are later shown in the anthracobioid types. Mycolachnea represents a important connection between the aleurioid and anthracobioid types, and at the same time, it also shows a similarity to the helvelloid type.

The ultrastructural studies of septal ontogeny in Morchellaceae and Sarcoscyphaceae are not yet completed. Once they are finished, the relationship between these families and evolutionary trends will be much clearer than at present.

This study suggests that all of the species studied in Humariaceae can be reorganized into four subgroups based on the ultrastructural data of spore ontogeny and septal structures (Tables 7.1 and 7.3). The first group is called

the "scutellinioid-GC" group, because they all share the scutellinioid septal type and produce ascospores by gradual condensation. These characters link Scutellinia, Cheilymenia, and Coprobia together. They grow on dung or rotten wood covered by mosses. The coprophilous species produce smooth to verrucose spores, while others are usually ornamented. All species are brightly pigmented and mostly covered by rooting hairs in their excipular tissues. This group is same as that recognized as Ciliarieae by Rifai (1968). This is the only tribe whose light microscope characters correlate consistently with ultrastructural data.

The second group is called the "anthracobioid-GC" group, because of their anthracobioid type of septa and gradual condensation type of spore ontogeny . This group is very close to the scutellinioid-GC group and accommodates Anthracobia, Caloscypha, Octospora, Otidea, Pyronema, and Sphaerosporella. Because of the presence of a translucent zone in the perisporic sac, many species have the potential of producing a spore sheath and smooth spores as in species of Cheilymenia. However, their apothecia are not covered by rooting excipular hairs and most of the genera have pyrophilic species.

The third group is called the "aleurioid-GC" group, because they have the aleurioid septal type and the gradual

condensation spore ontogeny type. A prominent feature of this group is the production of ornamented ascospores. The ornaments are ususally obvious and well constructed, even though the sporal ornaments of Trichophaea species are easily dissolved in 3% KOH.

The fourth group is called "helvelloid-DP" group, because of their helvelloid-like septal pore plugs and "direct precipitation" spore ontogeny types. Species of the two genera placed here, Geopyxis and Pulvinula, are pyrophilic or possibly mycorrhizal. This group is distinguished from other Humariaceae by their direct precipitation type of spore ontogeny. Their ascospores are either smooth or minutely echinulate. Although they show the helvelloid septal types, their spore and apothecial characters differ greatly from members of the Helvellaceae.

It is worthy to note that traditional characters such as carotenoid pigments, or excipular hairs, are probably so variable and unstable that they can not be used as major criteria to separate Humariaceae into phylogenetically related subgroups. For instance, Aleuria, Anthracobia, Caloscypha, Geopyxis, Leucoscypha, Octospora, and Pulvinula are treated in the Aleurieae, because they all have bright carotenoid pigments and similar ecological habitats. However, they show polyphyletic characters in the septal and spore ontogeny types (Table 7.2). Types of spore

ontogeny and septal structures have been shown to be very consistent at the generic and familial levels (Curry and Kimbrough, 1983; Kimbrough and Curry, 1985; Dyby and Kimbrough, 1987; Kimbrough *et al.*, 1991).

A number of genera in Humariaceae are pyrophilic species and produce smooth spores. There are at least two different ways of producing smooth ascospores (Fig. 7.2). The first way is to produce spores by a gradual condensation of wall material, a pattern commonly found in pyrophilic species (Tables 7.1 and 7.4) and is characterized by the disintegration of the perisporic sac (Fig. 7.2A). The exposed outer surface is a naked layer of epispore. The second way is like that illustrated from Geopyxis (Fig. 7.2B) in which the wall is produced by a direct precipitation of secondary wall material. The spore surface is covered by a layer of secondary wall. Certainly, there are some pyrophilic species which can produce rough ascospores, such as Peziza echinospora Karst. (Rifai, 1968; Petersen, 1970, 1975); P. petersii Berk. & Curt. (Rifai, 1968; Carpenter *et al.*, 1982); P. praetervisa Bres. (Rifai, 1968; Carpenter *et al.*, 1982; Dyby and Kimbrough, 1987), etc. However, their spore ontogeny is only by direct precipitation type (Dyby and Kimbrough, 1987; unpublished data). Because of the absence of a translucent zone in the perisporic sac and the direct

precipitation of secondary wall material onto epispore, it is feasible for some of pyrophilic species to produce rough ascospores. Conclusively, there are three different types of ascospores produced by pyrophilic species, (i) epispore-exposed smooth spore, (ii) secondary wall-exposed smooth spore, and (iii) secondary wall-exposed ornamented spore (Table 7.5).

During this study, a number of phenomena have been observed from the pyrophilic taxa of Humariaceae and these all suggest that pyrophilic pezizalean species may play an important role in fungal evolution.

1. Pyrophilic species may be found among genera bearing each of the septal types,(Tables 7.1 and 7.3).
2. Pyrophilic species may form mycorrhizae or parasitize liverworts, mosses or higher plants after burning as has been confirmed by Warcup (1990). He inoculated some pyrophilic species onto Eucalyptus and Melaleuca and a successful mycorrhizal association was induced. This could also be supported from the fact that some truffles have the same types of septal structures as epigeous discomycetes, such as Hydnoboletis with the pezizoid type septum (Kimbrough, *et al.*, 1992), and Barssia with the helvelloid type septum (unpublished data).
3. After burning, soil pH is normally lower, around 4.0-5.0 and then goes up to about 7.0, when more organic matter

is leached out (Petersen, 1970, 1971). This provides a shock that is probably essential to spore germination in pyrophilic species (El-Abyad and Webster, 1968; Wicklow, 1975; Wicklow and Zak, 1979; Zak and Wicklow, 1978a, 1978b, 1980). This kind of shock is very similar to that found in coprophilous fungi when the spores pass through the mammal's intestinal canal and are excreted with the droppings. Both coprophilous and pyrophilous species are often isolated from burnt soil (Wicklow, 1975).

4. Since a translucent zone in the perisporic sac is only found in the coprophilous fungi and some pyrophilic species that produce smooth, epispore-exposed ascospores, high temperature habitats appear to be correlated with the formation of translucent zone and abortion of perisporic sac.
5. Species in the same genus may be pyrophilic or not, for example, Trichophaea abundans is pyrophilic, but T. paludosa is not. Yet, both species show the same type of spore ontogeny, but it is not necessary for all of them to produce smooth or rough ascospores. T. abundans produces smooth ascospores, but T. paludosa produces rough ascospores.
6. The simplest type of ascospore would probably be globse, haline, smooth, and thin-walled. Most globose spored

discomycetes are also pyrophilic species. The globose spored species were considered to be more primitive by Eckblad (1968). These include, for example, species of Boudiera, Lamprospora, Pulvinula, Plicaria, and Sphaerosporella. The other possible pyrophilic species are Marcellina atroviolacea and Caloscypha fulgens. Although I could not find a record that they were collected from burnt ground, they are commonly found among mosses. All of the globose spored species which are not pyrophilic could perhaps be recognized as groups that have subsequently adapted to soil habitats.

7. It is probable that the most primitive discomycete species are those that produce only globose spores, the more advanced with smooth, ellipsoid spores, and the most advanced or well developed species with ellipsoid highly ornamented spores.

The following evolutionary model is proposed to explain the phylogenetic relationship of four subgroups of Humariaceae (Table 7.3) with other families of Pezizales, and to further explain the seven phenomena mentioned above. Among these four subgroups, "anthracobioid-GC" group is considered comparatively primitive (Table 7.6), since they mostly produce smooth, epispore-exposed ascospores, and they like Pyronema, another member of this group, also produce very simple apothecia. From this group, there seem

to be two directions of evolution, one to the "scutellinioid-GC" group, then to Ascobolaceae, and the other to the "aleurioid-GC" group and "helvelloid-DP" group which connects both Helvellaceae and Morchellaceae (Fig. 7.5). The close relationship among anthracobioid, scutellinioid, and ascoboloid types has been discussed thoroughly in Chapter V and VI. Ascobolaceae is thought to be an advanced group for their complex spore ontogeny and specific purple-brown coloration. In the other direction, the hemispherical convex band in the septal plug disappears, instead more fan-shaped and amorphous plugs are present and associated with striated, electron translucent toruses by the pore margin. In this trend, more and more species produce either elaborately ornamented spores or spores covered with a smooth secondary wall.

"Scutellinioid-GC" group appears to connect another two families, Pezizaceae and Ascodesmidaceae, because the former has the structural foundation to create a biconvexed or convexed structures (Chapter VI) similar to those found in the latter two families. Iodophanus plays an important role in connecting "scutellinioid-GC" and "pezizoid-DP" groups, because intermediate characters of both groups have been found in this genus (unpublished data).

Without exception, pyrophilic species are found in each of the four groups of Humariaceae (Table 7.3). It is

hypothesized that pyrophilic species have emerged from mutation after burning. Before this mutation, a parental ancestor is speculated as an organism producing spores by gradual condensation type. This idea is based on the fact that lichenized fungi, a group believed to be ancestral to heterotrophic ascomycetes (Cain, 1972; Sherwood and Gray, 1985; Hawksworth, 1988) also produce spores by this type of spore wall deposition (Honnerger, 1985). Two mutations are suggested, one is in spore ontogeny type (from gradual condensation type to direct precipitation type) and the other is in septal structures. Once mutated species are selected, they try to associate with other plants as parasites or symbionts and survive (Warcup, 1990; Boullard, 1988; Döbbeler, 1979). If a mutant still keeps the "gradual condensation" spore ontogeny gene, even though the perisporic sac loses temporarily the ability to form ornamented spores, but after adaptation to soil habitat, it may revert and produce ornamented spores. Consequently, it is not surprising to find ornamented spored species and smooth spored species in the same genus. Among these four groups, only "anthracobioid-GC" group accommodates mostly pyrophilic species. Mutation from the "gradual condensation" type to the "direct precipitation" type seems to be irreversible, because it is never found in the same genus.

Table 7.1 Spore Ontogeny, Septal Structure and the genera studied in the Pezizales (Ascomycetes)

Genus	S.T.	S.O.
Ascobolus[p] *Saccobolus*	Ascoboloid	GC
Scutellinia[p] *Cheilymenia* *Coprobia*	Scutellinioid	GC
Anthracobia[p] *Caloscypha* *Octospora*[p] *Otidea* *Pyronema**[p] *Sphaerosporella*[p]	Anthracobioid	GC
Aleuria *Leucoscypha** *Mycolachnea*[p] *Trichophaea*[p]	Aleurioid	GC
Helvella *Geopyxis*[p] *Gyromitra* *Pulvinula*[p]	Helvelloid	DP
Ascodesmis *Coprotus** *Eleutherascus*	Ascodesmioid	DP
Peziza[p] *Iodophanus* *Pachyella** *Plicaria*[p]	Pezizoid	DP

G C : gradual condensation
D P : direct precipitation
S.T.: septal type
S.O.: spore ontogeny
* : unpublished data
p : pyrophilic or potentially pyrophilic species.

Table 7.2 Selected genera of Humariaceae in Rifai's classification system

Tribe	Genera	S. T.	S. O.
	Aleuria	A-1	GC
	Anthracobia	A-2	GC
	Caloscypha	A-2	GC
Aleurieae	*Geopyxis*	H	DP
	Leucoschypha	A-1	GC
	Octospora	A-2	GC
	Pulvinula	H	DP
	Cheilymenia	S	GC
Ciliarieae	*Coprobia*	S	GC
	Scutellinia	S	GC
	Mycolachnea	T	GC
Lachneae	*Sphaerosporella*	A-2	GC
	Trichophaea	A-1	GC
Otideae	*Otidea*	A-2	GC
	Tarzetta	?	DP

A-1 : aleurioid type
A-2 : anthracobioid type
T : aleurioid/anthracobioid/helvelloid transitonal type
S : scutellinioid type
H : helvelloid type
G C : gradual condensation type
D P : direct precipitation type
S.T.: septal type
S.O.: spore ontogeny type

Table 7.3 The common characters found in four subgroups of Humariaceae, after this study

Genus	S.S.	T.Z.	S.W.	KOH RX/S.W.	R.H.
*Scutellinia**	+	+	R/S	-	+
Cheilymenia	+	+	S	-	+
Coprobia	+	+	S	-	-
*Anthracobia**	+	+	S	-	-
Caloscypha	+	+	S	-	-
*Octospora**	+	+	R/S	+[2]	-
Otidea	+/-	+	S[1]	-	-
*Pyronema**	+	+	S	-	-
*Sphaerosporella**	+	+	S	-	-
Aleuria	-	-	R	-	-
Leucoscypha	-	-	R	-	-
*Mycolachnea**	-	-	R	-	-
*Trichophaea**	-	+/-	R/S	+[2]	-
*Geopyxis**	-	-	S	-	-
*Pulvinula**	-	-	S[3]	-	-

S.S.: spore sheath
T.Z.: translucent zone
S.W.: spore wall
R.H.: rooting hairs in excipular tissue
KOH RX/S.W.: KOH reaction with spore wall
R: rough spore surface
S: smooth spore surface
1: spore surface is covered by fragile ridges, not visible by light microscope.
2: spore surface is covered by rough ornaments but easily dissolved in KOH and becomes smooth.
3: spore surface is covered by minute echinulation, not visible at light microscope.
*: pyrophilic or potentially pyrophilic species

Table 7.4 The genera could produce globose and smooth spores[3]

Genera	G.S.	S.W.	S.O.	pyrophilic
Pithya	+	-	?	-
Boedijnopeziza	+	+	?	-
Pseudoplectania	+	+	?	-
Gyromitra	+	+	DP	-
Ascobolus	+	+	GC	+
Boudiera	+	+	?	+
Sphaerosoma	+	-	?	+
Sphaerosporella	+	+	GC	+
Ascodesmis	+	-	DP	-
Pulparia[1]	+	+	?	+
Sphaerozone	+	-	?	+
Scutellinia	+	+	GC	+*
Caloscypha	+	+	GC	-
Pulvinula[1]	+	+	DP	+
Lamprospora	+	+	?	+*
Peziza[2]	+	+	DP	+*

Remarks: 1: *Pulparia=Pulvinula=Marcellina=? Greletia*
2: including *Plicaria*
3: data after Korf (1972)
*: producing both globose and ellipsoid spores.
G.S.: globular spore
S.W.: smooth wall
S.O.: spore ontogeny type
G C : gradual condensation type
D P : direct precipitation type

Table 7.5 The spore wall characters found in pyrophilic speices

Spore Wall	S. O.	Rough	Smooth
Epispore exposed	GC	-	+
S.W. exposed	DP	+	+

S.O.: spore ontogeny type
S.W.: secondary wall
G C : gadual condensation type
D P : direct precipitation type

Table 7.6 Some of phylogenetic characters in Humariaceae

Groups	R.S.	S.S.	E.S.	B.S.	GC/DP	T.Z.	E.H.	R.H.
"Scut-GC"	1	0	0	0	0	0	1	1
"Anth-GC"	1	0	0	0	0	0	1	0
"Aleu-GC"	1	1	0	0	0	1	1	0
"Helv-DP"	1	1	1	0	1	1	0	0

R.S.: rough spore
S.S.: smooth spore
E.S.: epispore-exposed smooth spore
B.S.: brown spore
G C : gradual condensation
D P : direct precipitation
T.Z.: translucent zone
E.H.: excipular hairs
R.H.: rooting hair
0 : plesiomorphy
1 : apomorphy

Figure 7.1 Comparative spore ontogeny between gradual condensation (A and B) and direct precipitation (C) types. B represents a typical sporogenesis in pyrophilic species.

a: outer investing membrane.

b: inner sporoplasma membrane.

c: perisporic sac.

d: primary wall.

e: dense bump of secondary wall.

f: spinelike precursors of epispore.

g: condensed granules.

h: ornaments.

i: translucent zone.

j: radiate structures in primary wall.

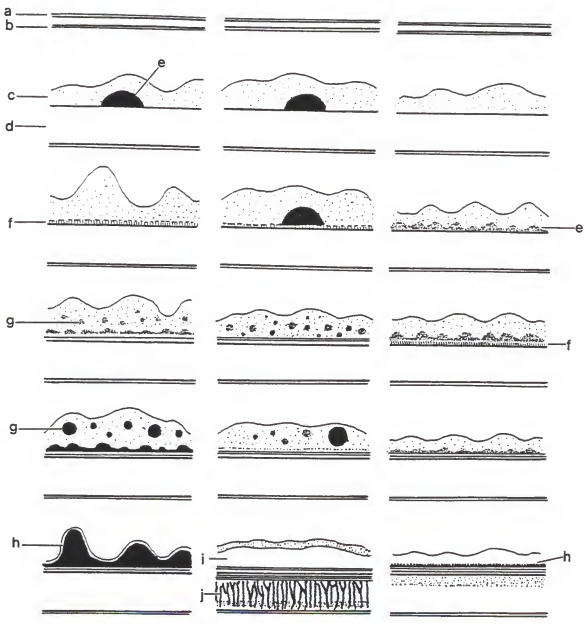

a
b
c
d
e
f
g
g
h
i
j
e
f
h
A
B
C
1

Figure 7.2 Comparative formation of smooth spore. A: gradual condensation type. B: direct precipitation type.

a: outer investing membrane

b: inner sporoplasma membrane

c: perisporic sac

d: primary wall

e: precursors of epispore

f: epispore

g: translucent zone

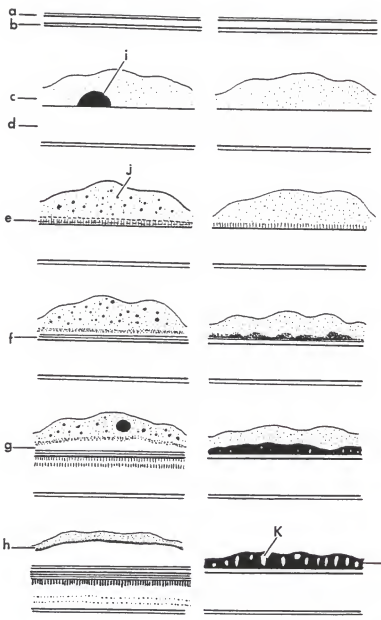
h: aborted perisporic sac

i: clump of secondary wall material

j: condensed granule

k: lacunae

l: secondary wall



2

Figure 7.3 Comparative spore ontogeny with different types of septal structures. The letters in parenthesis are representatives of the genus depicted in the figure.

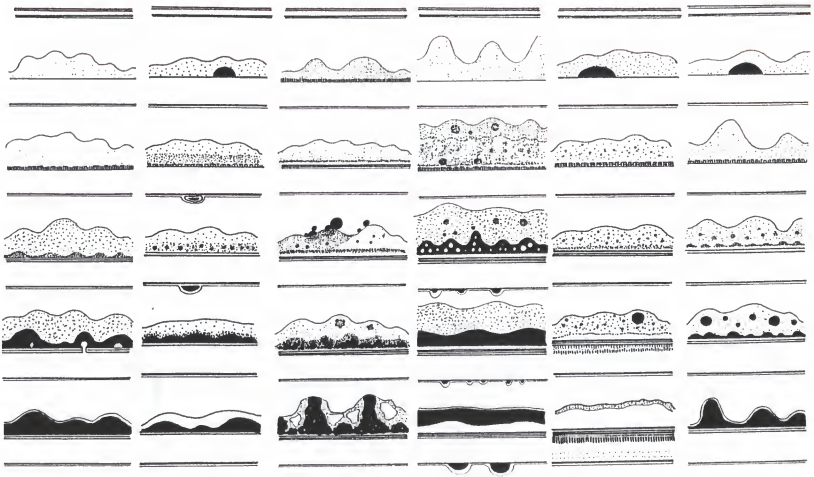

Pezizoid
Helvelloid
Mycolachnea
Ascoboloid
Scutellinioid
(Cheilymenia)
Anthracobioid
(Sphaerosporella)
Aleurioid

Figure 7.4 Comparative septal developments between in ascogenus hyphae (A) and ascal bases (B).

A-1: ascoboloid type.

A-2: scutellinioid type.

A-3: anthracobioid type.

A-4: transitional type of Mycolachnea.

A-5: aleurioid type.

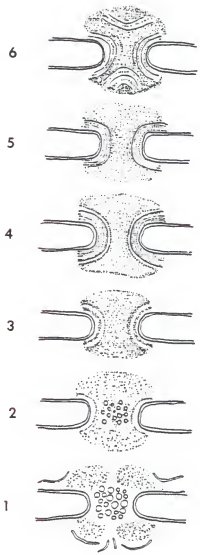
A-6: helvelloid type.

B-1: ascoboloid/scutellinioid/anthracobioid types.

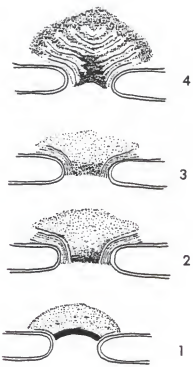
B-2: transitional type of Mycolachnea.

B-3: aleurioid type.

B-4: helvelloid type.



A



B

(4)

Figure 7.5 Phylogenetic arrangement of Humariaceae and other families in Pezizales, based on septal development and spore ontogeny data.

A: ascoboloid-GC type.

B: scutellinioid-GC type.

C: anthracobioid-GC type.

D: transitional type from Mycolachnea hemisphaerica.

E: helvelloid-DP type from Pulvinula convexella.

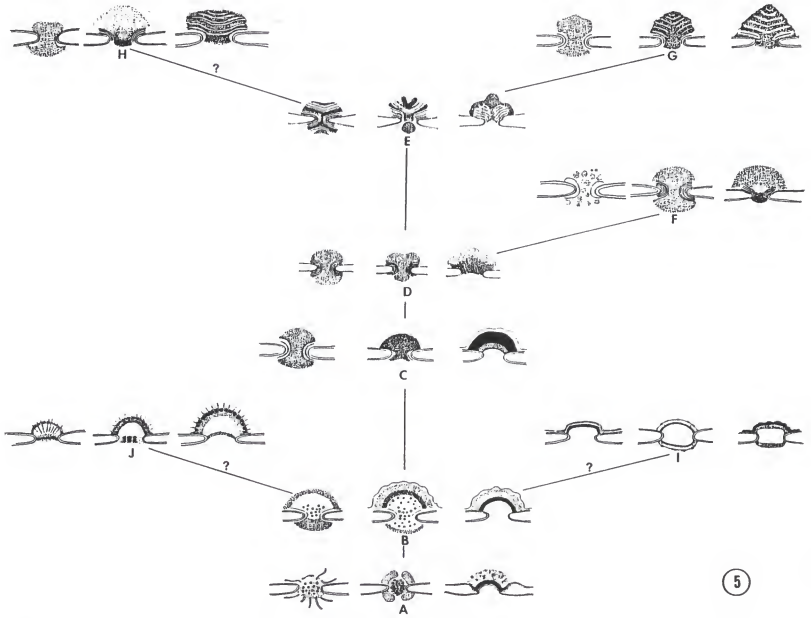
F: aleurioid-GC type.

G: helvelloid-DP type from Helvella.

H: Morchella (septal type is uncertain).

I: pezizoid-DP type (septal development is uncertain).

J: ascodesmioid type (septal development is uncertain).



(5)

SUMMARY

1. Apothecial morphology, presence or absence of carotenoid pigment, excipular hairs, spore ornamentation, and guttules are characters more subject to environmental pressures and should not be given first priority in organizing operculate discomycetes into tribes or families.
2. Two basic types of spore ontogeny are found in Pezizales, (i) gradual condensation (GC) and direct precipitation (DP).
3. More than 7 different types of septal structures exist in Pezizales and four different types are found in Humariaceae..
4. At least four groups of fungi can be distinguished based on septal and spore ontogeny types. They are (i) scutellinioid-GC group, including Scutellinia, Cheilymenia, and Coprobia producing rough or smooth ascospores; (ii) anthracobioid-GC group, including Anthracobia, Caloscypha, Octospora, Otidea, Pyronema, and Sphaerosporella, producing mostly smooth ascospores; (iii) aleurioid-GC group, including Aleuria, Leucoscypha, Mycolachneae, and Trichophaea, producing mostly rough ascospores; (iv) helvelloid-DP group,

including Geopyxis, and Pulvinula, producing smooth ascospores.

5. The development of septal structures may provide the key connection between families in the Pezizales.
6. A specific septal type always correlates with a distinct type of spore ontogeny.
7. Pyrophilic species probably plays an important role in the fungal evolution.
8. New familial definitions to reorganize the genera of Humariaceae are needed.

BIBLIOGRAHPY

Arpin, N. 1968. Les caroténoides de discomycètes: Essai chimiotaxonomique. Theses 527. Univ. Lyon. 170 pp. Villeurbanne, France.

Batra, L. R. 1960. A preliminary study of the Indian discomycete flora. Mimeographed. Swarthmore College, Swarthmore, Pennsylvania.

Batra, L. R., and S. W. T. Batra. 1963. Indian Discomycetes. In Univ. Kansas Sci. Bull. 44: 109-256.

Bellèmere, A., and L.-M. Melendez-Howell. 1976. Etude ultrastructurale comparee de l'ornementation externe de la paroi des ascospores de delux Pezizales:Peziza fortini n.sp., recoltee au mexique, & Aleuria aurantia (Oed. ex Fr.) Fuck. Revue de Mycologie 40:3-19.

Benny, G. L., and D. A. Samuelson. 1980. Ultrastructure of septa in the vegetative hyphae of Chaetomidium arxii and Phillipsia domengensis (Ascomycotina). Mycologia 72:836-840.

Berthet, P. 1964. Éssai biotaxonomique sur des Discomycétes. Ph.D. thése, Université Lyon, France.

Berthet, P., and R. P. Korf. 1969. Sur la position taxonomique du genre Jafnea Korf. Naturaliste Canad. 96:247-249.

Blanchard R. O. 1972. Septa in Sporormia australis. Mycologia 64:1330-1333.

Boudier, J. L. E. 1885. Nouvelle classification naturelle des discomycètes charnus connus généralement sous le nomde pezizes. Bull. Soc. Mycol. Fr. 1: 91-120.

Boudier, J. L. E. 1907. Histoire et classification des Discomycètes d'Europe. I-VII: 1-223. P. Klincksich, Paris.

Boullard, B. 1988. Observations on the coevolution of fungi with hepatics. *In* K. A. Pirozynski and D. L. Hawksworth, Coevolution of fungi with plants and animals", pp 107-124, Academic Press, London.

Breitenbach, J., and F. Kränzlin. 1984. Fungi of Switzerland Vol. I: ascomycetes. Verlag Mykologia, Luzern, Switzerland.

Bronchart, R., and V. Demoulin. 1975. Septum ultrastructure of Ostracoderma torrendii. Can. J. Bot. 53:1549-1553.

Brummelen, J. Van 1981. The operculate ascus and allied forms. In Don R. Reynolds, Ascomycete Systematics. Springer-Verlag, New York.

Brummelen, J. Van 1989. Ultrastructure of the ascus and the ascospore wall in Eleutherascus and Ascodesmis (Ascomycotina). Persoonia 14: 1-17.

Cain, R. F. 1972. Evolution of the Fungi. Mycologia 64:1-14.

Camp, R. R. 1977. Association of microbodies, Woronin bodies, and septa in intercellular hyphae of Cymadothea trifolii. Can. J. Bot. 55:1865-1859.

Carpenter, S. E., J. M. Trappe, and G. A. Hunt. 1982. Observations on fungal succession on recent volcanic deposits of mount St. Helens. Oregon Academy of Science, Oregon St. Univ. 15: 36-44.

Carroll, G. C. 1966. A study of the fine structure of ascosporogenesis in Saccobolus kervernii and Ascodesmis sphaerospora. Ph.D. thesis, University of Texas, Austin.

Carroll, G. C. 1969. A study of the fine structure of ascosporogenesis in Saccobolus kervernii. Arch. Mikrobiol. 66:321-339.

Cole, G. T., and H. C. Aldrich. 1971. Ultrastructure of conidiogenesis in Scopulariopsis brevicaulis. Can. J. Bot. 49:745-755.

Curry, K. J., and J. W. Kimbrough. 1983. Septal structures in apothecial tissues of the Pezizaceae (Pezizales, Ascomycetes). Mycologia 75:781-794.

Delay, C. 1966. Étude de l'infrastructure de l'asque d'Ascobolus immersus pers. pendant la maturation des spores. Ann. Sci. Natur. (Bot.) 7:361-420.

Denison, W. C. 1959. Some species of the genus Scutellinia. Mycologia 51: 605-635.

Denison, W. C. 1964. The genus Cheilymenia in North America. Mycologia 61: 718-737.

Dennis, R. W. G. 1968. British Ascomycetes. 455 pp. J. Cramer, Lehre.

Dennis, R. W. G. 1978. British ascomycetes. J. Cramer. Valduz. 585 pp.

Döbbeler, P. 1979. Untersuchungen an moosparasitischen Pezizales aus der Verwandtschaft von Octospora. Nova Hedwigia 31:817-864.

Dyby, S., and J. W. Kimbrough. 1987. A comparative ultrastructural study of ascospore ontogeny in selected species of Peziza (Pezizales; Ascomycetes). Bot. Gaz. (Crawfordsville) 148:283-296.

Eckblad, F.-E. 1968. The genera of the operculate discomycetes. A reevaluation of their taxonomy, phylogeny and nomenclature. Nytt. Mag. Bot. 15:1-191.

El-Abyad, M. S. and J. Webster. 1968. Studies on pyrophilous discomycetes. I. Comparative physiological studies. Mycologia 51:353-367.

Fries, E. M. 1823. Systema mycologicum. Vol. 2(1):1-274. Berlingiana, Lund.

Gibson, J. L., and J. W. Kimbrough. 1988a. Ultrastructural observations on Helvellaceae (Pezizales). I. Ascosporogenesis of selected species of Helvella. Can. J. Bot. 66:771-783.

Gibson, J. L., and J. W. Kimbrough. 1988b. Ultrastructural observations on Helvellaceae (Pezizales). II. Ascosporogenesis of Gyromitra esculenta. Can. J. Bot. 66:1743-1749.

Hammill, T. 1974. Septal pore structure in Trichoderma saturnisporum. Amer. J. Bot. 61:767-771.

Hallgrímsson and H. F. Gøtzsche. 1990. Notes on Ascomycetes II: Discomycetes. Acta Bot. Isl. 10:31-36.

Harmaja, H. 1974a. The generic limit between Otidea and Tarzetta (Pustularia auct.). Karstenia 14: 138-142.

Harmaja, H. 1974b. Flavoscypha, a new genus of the Pezizales for Otidea cantharella and O. phlebophora. Karstenia 14:105-108.

Harmaja, H. 1974c. Tarzetta pusilla n. sp. and T. spurcata (Pers.) n. comb. from Finland. Karstenia 14:116-120.

Harmaja, H. 1974d. Observations on the presence of acyanophilic perispore in the spores of the Pezizales. Karstenia 14:123-125.

Hawksworth, D. L. 1988. Coevolution of fungi with algae and cyanobacteria in lichen symbioses. *In* K. A. Pirozynski and D. L. Hawksworth, Coevolution of fungi with plants and animals, pp 125-148, Academic Press, London.

Hawksworth, J. C. D. 1989. Family names, Index of Fungi (Suppl.). CAB, IMI, Kew, Surrey, UK., 75pp.

Heath, I. Brent. 1978. Experimental studies of mitosis in the fungi. *In* I. Brent Heath, Nuclear division in the fungi, pp. 89-176, Academic Press, London.

Honegger, R. 1985. Ascus structure and ascospore formation in the lichen-forming Chaenotheca chrysocephala (Caliciales). Sydowia 38:146-157.

Janex-Favre, M. C. and A. PargueyLeduc. 1980. Formation et èvolution des ascospores du Tuber mesentericum Vitt. Bull. Soc. Mycol. Fr. 96:225-237.

Janex-Favre, M. C. and A. PargueyLeduc. 1983. Étude ultrastructurale des asques et des ascospores de truffes du genre Tuber. II. Les ascospores. Cryptogamie, Mycol. 4:353-373.

Janex-Favre, M. C. and A. PargueyLeduc. 1985. Les asques et les ascospores de Terfezia claveryi Ch. (Tubèrales). Cryptogamie, Mycol. 6:87-99.

Janex-Favre, M. C. and A. PargueyLeduc. 1988. Les asques des Tuber (Discomycètes, Tubèrales): particularités morphologiques et structurales. pp 111-120. Secondo Congresso internazionale sul Tartufo, Spoleto 24-27 Novembre 1988.

Janex-Favre, M. C., A. Parguey-Leduc, and L. Riousset. 1988. L'ascocarpe hypoge d'une terfez francaise (Terfezia leptoderma Tul., Tuberales, Discomycetes). Bull. Soc. Myc. Fr. 104(3):145-178.

Kanouse, B. B. 1958. Some species of the genus Trichophaea. Mycologia 50: 121-140.

Kanouse, B. B. 1949. Studies on the genus Otidea. Mycologia 41:661-677.

Khan, Saeed R., and H. C. Aldrich. 1975. The haustoria of Termitaria snyder. J. Invert. Pathol. 25:247-260.

Kimbrough, J. W. 1970. Current trends in the classification of discomycetes. Botan. Rev. 36:91-161.

Kimbrough, J. W. 1989. Arguments towards restricting the limits of the Pyronemataceae (Ascomycetes, Pezizales). Mem. New. York. Bot. Gard. 49:326-335.

Kimbrough, J. W. 1990. Septal structures and systematics of operculate discomycetes. (Abstract) 4th Int. Mycol. Cong. Regensburg, Germany.

Kimbrough, J. W. 1991. Ultrastructural observations on Helvellaceae (Pezizales, Ascomycetes). V. Septal structures in Gyromitra. Mycol. Res. 95(4):421-426.

Kimbrough, J. W., and K. J. Curry. 1985. Septal structure in the Ascobolaceae (Pezizales, Discomycetes). Mycologia 77: 219-229.

Kimbrough, J. W., and K. J. Curry. 1986a. Septal structures in apothecial tissues of taxa in the tribes Scutellinieae and Sowerbyelleae (Pyronemataceae, Pezizales, Ascomycetes). Mycologia 78: 735-743.

Kimbrough, J. W., and K. J. Curry. 1986b. Septal structures in apothecial tissues of the tribe Aleurieae in the Pyronemataceae (Pezizales, Ascomycetes). Mycologia 78(3):407-417.

Kimbrough, J. W., and J. L. Gibson. 1989. Ultrastructural observations on Helvellaceae (Pezizales; Ascomycetes). III. Septal structures in Helvella. Mycologia 81: 914-920.

Kimbrough, J. W., and J. L. Gibson. 1990. Ultrastructural and cytological observations of apothecial tissues of Geopyxis carbonaria (Pezizales, Ascomycetes). Can. J. Bot. 68:243-257.

Kimbrough, J. W., C.-G. Wu, and J. L. Gibson. 1992. Ultrastructural evidence for a phylogenetic linkage of the truffle genus Hydnobolytis to the Pezizaceae (Pezizales, Ascomycetes). Bot. Gaz. (in press).

Kimbrough, J. W., C.-G. Wu, and J. L. Gibson. 1990. Ultrastructural observations on Helvellaceae (Pezizales, Ascomycetes). IV. Ascospore ontogeny in selected species of Gyromitra subgenus Discina. Can. J. Bot. 68:317-328.

Korf, R. P. 1973. Discomycetes and Tuberales. *In* Ainsworth, G. C., F. K. Sparrow, and A. S. Sussman. The Fungi IVa pp. 249-319. Academic Press, New York.

Korf, R. P., and W.-Y. Zhuang. 1984. The ellipsoid spored species of Pulvinula (Pezizales). Mycotaxon 20(2): 607-616.

Le Gal, M. 1947. Researches sur les ornamentations sporales des discomycetes opercules. Ann. Sci. Nat., Bot. sér. 11: 73-297.

Le Gal, M. 1953. Les Discomycètes de Madagascar. Laboratoire de Cryptogmie du Museum National d'Histoire Naturelle, Paris. 465 p.

Littlefield L. J. and C. E. Bracker. 1972. Ultrastructure of septa in Melampsora lini. Trans. Br. mycol. Soc. 56:181-188.

Maire, R. 1937. Fungi Catalaunici. Series Altera. In Treb. Mus. Cienc. nat., Barcelona III 4: 1-128.

Merkus, E. 1973. Ultrastructure of the ascospore wall in Pezizales (Ascomycetes)-I. Ascodesmis microsporica (Crouan) Seaver and A. nigricans van Tiegh. Persoonia 7(3):351-366.

Merkus, E. 1974. Ultrstructure of the ascospore wall in Pezizales (Ascomycetes)-II. Pyronemataceae sensu Eckblad. Persoonia 8(1):1-22.

Merkus, E. 1975. Ultrastructure of the ascospore wall in Pezizales (Ascomycetes)-III. Otideaceae and Pezizaceae. Persoonia 8(3):227-247.

Merkus, E. 1976. Ultrastructure of the ascospore wall in Pezizales (Ascomycetes)-IV. Morchellaceae, Helvellaceae, Rhizinaaceae, Thelebolaceae and Sarcoscyphaceae, general discussion. Persoonia 9(1):1-38.

Mims, C. W., E. A. Richardson, and J. W. Kimbrough. 1990. Ultrastructure of ascospore delimitation in freeze substituted samples of Ascodesmis nigricans (Pezizales). Protoplasma 156: 94-102.

Moore, E. J., and Richard P. Korf. 1963. The genus Pyronema. Bull. Torr. Bot. Club 90:33-42.

Moravec, Jiri 1988. Cheilymenia fraudans and remarks on the genera Cheilymenia and Coprobia. Mycotaxon 31: 483-489.

Moravec, Jiri 1990. Taxonomic revision of the genus Cheilymenia-3. A new generic and infrageneric classification of Cheilymenia in a new emendation. Mycotaxon 38: 459-484.

Pant, D. C. and V. P. Tewari. 1970. Observations on two species f the genus Pulvinula. Mycologia 62:1187-1194.

Parguey-Leduc, A., M. C. Janex-Favre, and C. Montant. 1987. Formation et évolution des ascospores de Tuber melanosporum (Truffe noire du Périgord, Discomycètes). Can. J. Bot.65:1491-1503.

Parguey-Leduc, A., M. C. Janex-Favre, and C. Montant. 1988. L'ascocarpe du Tuber melanosporum Vitt. (Truffe noire du Périgord, Discomycètes): structure de la glèbe. I. Les veines fertiles. pp 101-109. Secondo Congresso internazionale sul Tartufo, Spoleto 24-27 Novembre 1988.

Parguey-Leduc, A., M. C. Janex-Favre, and C. Montant. 1990. L'appareil sporophytique et les asques du Tuber melanosporum Vitt. (Truffe noire du Périgord, Discomycètes). Cryptogamie, Mycol. 11:47-68.

Pepin, R. 1971. Sur les ultrastructures associées aux pores septaux dans l'apothecie de Galactinia cerea (Sow. ex Merat) le Gal, champignon Discomycete. Compt. Rend. Hebd. Seances Acad. Sci. Sér. D 272:3126-3128.

Petersen, P. M. 1970. Danish fireplace fungi-An ecological investigation on fungi on burns. Dansk Bot. Ark. 27: 7-97.

Petersen, P. M. 1971. The Macromycetes in a burnt forest area in Denmark. Bot. Tidsskr. 66: 228-248.

Petersen, P. M. 1975. Fireplace Fungi in an artic area: middle west Greenland. FRIESia 10: 270-280.

Pfister, D. H. 1976. A synopsis of the genus Pulvinula. A new combination in the genus Gymnomyces. Farlow Herb. Harv. Univ. No. 9:1-19.

Reichle, R. E., and J. V. Alexander. 1965. Multiperforate septa, Woronin bodies and septal plugs in Fusarium. J. Cell Biol. 24:489-496.

Rifai, M. A. 1968. The Australasian Pezizales in the herbarium of the Royal Botanical Garden Kew. Verh. Kon. Ned. Akad. Wetensch. Afd. Natuurk. Tweede Sect. II. 57:1-295.

Samuelson, D. A. 1977. Asci of the operculate discomycetes (Pezizales). Ph.D thesis, University of Florida, Gainesville.

Samuelson, D. A. 1978a. Asci of the Pezizales. I. The apical apparatus of iodine-positive species. Can. J. Bot. 56:1860-1875.

Samuelson, D. A. 1978b. Asci of the Pezizales-II. The apical apparatus of representatives in the Otidea-Aleurea complex. Can. J. Bot. 56:1876-1904.

Samuelson, D. A. 1978c. Asci of the Pezizales-III. The apical apparatus of eugymnohymenial representatives. Am. J. Bot. 65:748-758.

Schrantz, J.-P. 1964. Étude au microscope électronique des synapse de deux Discomycetes. Compt. Rend. Hebd. Seances Acad. Sci. sér. D 258:3342-3344.

Schrantz, J.-P. 1966. Contribution à l'étude de la formation de la paroi sporale chez Pustularia cupularis (L.) Fukl. C. r. Acad. Sci., Paris 262:1212-1215.

Schrantz, J.-P. 1970. Étude cytologique en microscopie optique et électronique, de quelques Ascomycetes. II. La paroi. Rev. Cytol. Biol. Veg. 33:111-168.

Schrantz, J.-P. 1971. Étude cytologique en microscopie optique et électronique de quelques Ascomycetes. III. Le cytoplasme. Rev. Cytol. et Biol. veg. 34:1-48.

Schumacher, T. 1979. Notes on taxonomy, ecology, and distribution of operculate discomycetes (Pezizales) from river banks in Norway. Norw. J. Bot. 26: 53-83.

Seaver, F. J. 1942. The North American cup-fungi (operculates). Suppl. ed. Seaver, New York. 377p.; *reprinted* 1961. Hafner, New York.

Sherwood, M. A. and J. Gray. 1985. Silurian fungal remains: probable records of the class Ascomycetes. Lethaia 18:1-20.

Warcup, J. H. 1990. Occurrence of ectomycorrhizal and saprophytic discomycetes after a wild fire in a eucalypt forest. Mycol. Res. 94:1065-1069.

Wells, K. 1972. Light and electron microscopic studies of Ascobolus stercorarius II. Ascus and ascospore ontogeny. Univ. Calif. Publs. Bot. 62:1-93.

Wicklow, D. T. 1975. Fire as an environmental cue initiating ascomycete development in a tall grass prairie. Mycologia 67:852-862.

Wicklow, D. T., and J. C. Zak. 1979. Ascospore germination of carbonicolous ascomycetes in fungistatic soils: an ecological interpretation. Mycologia 71:238-242.

Wu, Chi-Guang. 1990. Ascosporogenesis in selected genera of Humariaceae (Pezizales, Ascomycetes) (summary). S. E. E. M. S. Newsletter 6(1): 9.

Wu, Chi-Guang, and James W. Kimbrough. 1990. Comparative ultrastructure of spore ontogeny in the Humariaceae (Pezizales). Mycol. Soc. Amer. Newsletter 41:44 (abstract).

Wu, Chi-Guang, and James W. Kimbrough. 1991a. Ultrastructural studies on ascosporogenesis in Ascobolus stictoideus (Pezizales, Ascomycetes). Can. J. Bot. 70: xx-xx.

Wu, Chi-Guang, and James W. Kimbrough. 1991b. Ultrastructural studies on ascosporogenesis in Ascobolus immersus (Pezizales, Ascomycetes). Mycologia 84:xx-xx (in press).

Zak, J. C., and D. T. Wicklow. 1978a. Response of carbonicolous ascomycetes to aerated steam temperatures and treatment intervals. Can. J. Bot. 56:2313-2318.

Zak, J. C., and D. T. Wicklow. 1978b. Factors influencing patterns of ascomycete sporulation following simulated "burning" of prairie soils. Soil Biol. Biochem. 10:533-535.

Zak, J. C., and D. T. Wicklow. 1980. Structure and composition of a post-fire ascomycete community: Role of abiotic and biotic factors. Can. J. Bot. 58:1915-1922.

BIOGRAPHICAL SKETCH

Chi-Guang Wu was born on September 14, 1957, in Nan-kan, Tao-yuang, Taiwan, Republic of China. He finished primary school and junior middle school education at Nan-kan, Tao-Yuang, in 1970 and 1973, respectively. In September of 1973, he entered Jian-kuo High School, Taipei, and in 1977, he passed the federal entrance examination for university and was admitted to National Taiwan University, Taipei. When he was a junior student in the Botany Department, he entered the Mycology Lab and began his mycological career. He earned his B.S. degree in 1981. Two years later, he finished a thesis on the vesicular-arbuscular mycorrhizal study in bamboo forest and was granted a M.S. degree. In the summer of 1983, he joined the military service and served in the Chinese Air Force for two years. In the summer of 1985, motivated by a tremendrous interest in VAM fungi, he passed another entrance examination for the Ph.D. program and kept investigating Endogonaceae in Taiwan. He withdrew from graduate school in the spring of 1986 and worked as a research assistant in the National Taiwan University. In April of 1987, he was married to Hwung Zue and in May, he was admitted to University of Florida, Plant Pathology

Department. Two years later, his son, James F. Wu, was born in Shands Hospital. He is a member of the American Mycological Society, Southeastern Electron Microscope Society (SEEMS), and Electron Microscope Society of America. In 1990, he received the Ruska Award from SEEMS for the best student research paper presented at the annual meeting.

I certify that I have read this study and that in my opinion it confirms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

James W. Kimbrough, Chairman
Professor of Plant Pathology

I certify that I have read this study and that in my opinion it confirms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

L. H. Purdy
Professor of Plant Pathology

I certify that I have read this study and that in my opinion it confirms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Harold C. Kistler
Assistant Professor of Plant Pathology

I certify that I have read this study and that in my opinion it confirms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Henry C. Aldrich
Professor of Microbiology and Cell Science

I certify that I have read this study and that in my opinion it confirms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Saeed R. Khan
Assistant Professor of
Pathology and Laboratory
Medicine

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

August, 1991

Dean, College of Agriculture

Dean, Graduate School